CUK- H03742-53-P024740

OLUME 110 (1) — JANUARY 2001

VOL-110
NO. 1-3
2001

53

— Founded in 1892 —

ANNALS

OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

Official Organ of the
AMERICAN LARYNGOLOGICAL ASSOCIATION
AMERICAN BRONCHO-ESOPHAGOLOGICAL ASSOCIATION

OMYCIN: EFFECTS ON LARYNGEAL AND ACHEAL STENOSIS, BENEFITS, AND COMPLICATIONS

UIRED TOTAL (GRADE 4) SUBGLOTTIC STENOSIS IN CHILDREN

AOPERATIVE ASSESSMENT OF STAPES MOVEMENT

GENITAL NASAL PYRIFORM APERTURE NOSIS: DIAGNOSIS AND MANAGEMENT OF 20 CASES

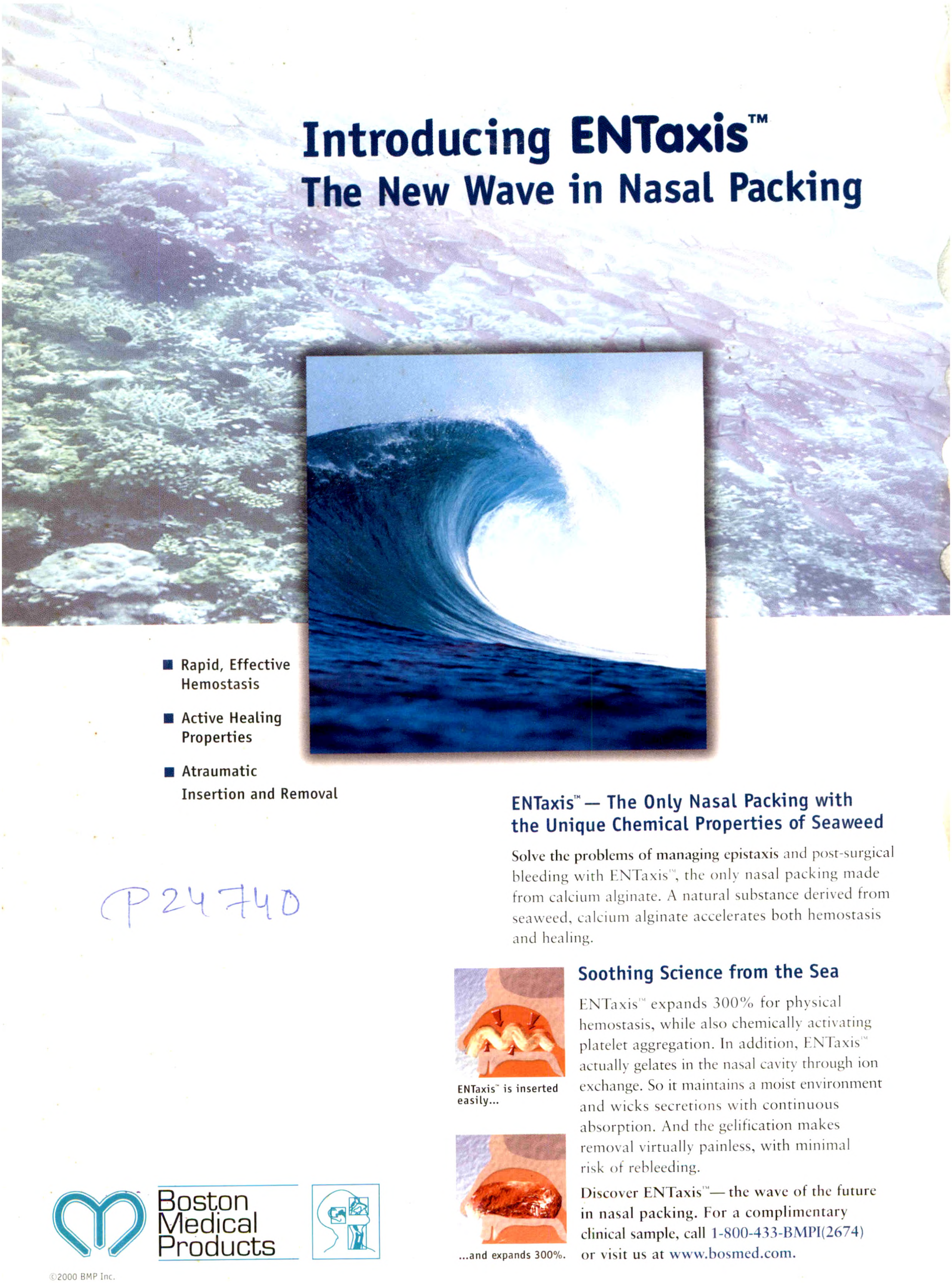
RFORMANCE CHARACTERISTICS OF RESCENCE ENDOSCOPE IN DETECTION OF HEAD AND NECK CANCERS

41

UNIVERSITY ... LIBRARY

WEB SITE—http://www.Annals.com

ANNALS
PUBLISHING CO.



Introducing ENTaxis™
The New Wave in Nasal Packing

- Rapid, Effective Hemostasis
- Active Healing Properties
- Atraumatic Insertion and Removal

ENTaxis™ — The Only Nasal Packing with the Unique Chemical Properties of Seaweed

Solve the problems of managing epistaxis and post-surgical bleeding with ENTaxis™, the only nasal packing made from calcium alginate. A natural substance derived from seaweed, calcium alginate accelerates both hemostasis and healing.

Soothing Science from the Sea

ENTaxis™ expands 300% for physical hemostasis, while also chemically activating platelet aggregation. In addition, ENTaxis™ actually gelates in the nasal cavity through ion exchange. So it maintains a moist environment and wicks secretions with continuous absorption. And the gelification makes removal virtually painless, with minimal risk of rebleeding.

Discover ENTaxis™— the wave of the future in nasal packing. For a complimentary clinical sample, call 1-800-433-BMPI(2674) or visit us at www.bosmed.com.

ENTaxis™ is inserted easily...

...and expands 300%.

Boston Medical Products

©2000 BMP Inc.

P24740

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

— Founded in 1892 —

53

Editor

BRIAN F. MCCABE, MD
Iowa City, Iowa

Associate Editors

CHARLES D. BLUESTONE, MD
Pittsburgh, Pa

GEORGE A. GATES, MD
Seattle, Wash

OLLIVIER LACCOURREYE, MD
Paris, France

Board of Editors

JOHN G. BATSAKIS, MD .... Houston, Tex; Kalkaska, Mich
JOEL M. BERNSTEIN, MD, PHD .... Buffalo, NY
ANDREW BLITZER, MD, DDS .... New York, NY
DERALD E. BRACKMANN, MD .... Los Angeles, Calif
POUL BRETLAU, MD, DMSC .... Copenhagen, Denmark
ITZHAK BROOK, MD .... Washington, DC
RICHARD A. BUCKINGHAM, MD .... Park Ridge, Ill
RICHARD A. CHOLE, MD, PHD .... St. Louis, Mo
ALFIO FERLITO, MD .... Udine, Italy
UGO FISCH, MD .... Zurich, Switzerland
IMRE FRIEDMANN, MD, SCD .... Middlesex, England
BRUCE GANTZ, MD .... Iowa City, Iowa
LEE A. HARKER, MD .... Omaha, Neb
JEFFREY P. HARRIS, MD, PHD .... San Diego, Calif
GERALD B. HEALY, MD .... Boston, Mass
WILLIAM F. HOUSE, MD .... Los Angeles, Calif
STEVEN K. JUHN, MD .... Minneapolis, Minn
TIMOTHY T. K. JUNG, MD, PHD .... Loma Linda, Calif
DAVID W. KENNEDY, MD .... Philadelphia, Pa
FRANK E. LUCENTE, MD .... New York, NY
WILLIAM L. MEYERHOFF, MD, PHD .... Dallas, Tex
JOSEF MILLER, PHD .... Ann Arbor, Mich
AAGE R. MØLLER, PHD .... Dallas, Tex
WILLIAM W. MONTGOMERY, MD .... Boston, Mass
EUGENE N. MYERS, MD .... Pittsburgh, Pa
JOSEPH B. NADOL, JR, MD .... Boston, Mass
YASUYA NOMURA, MD .... Tokyo, Japan
LORNE S. PARNES, MD .... London, Canada
JAI H. RYU, PHD .... Winston-Salem, NC
JACOB SADÉ, MD .... Tel Aviv, Israel
ISAMU SANDO, MD .... Pittsburgh, Pa
CLARENCE T. SASAKI, MD .... New Haven, Conn
ROBERT T. SATALOFF, MD, DMA .... Philadelphia, Pa
RICHARD J. H. SMITH, MD .... Iowa City, Iowa
HEINZ R. STAMMBERGER, MD .... Graz, Austria
DEAN M. TORIUMI, MD .... Chicago, Ill
JAN E. VELDMAN, MD, PHD .... Utrecht, The Netherlands
BRUCE M. WENIG, MD .... Bronx, NY
T. J. YOO, MD, PHD .... Memphis, Tenn

Software Review and Computer Applications Editor

KEVIN T. KAVANAGH, MS, MD .... Somerset, Ky

Imaging Editor

YUTAKA SATO, MD .... Iowa City, Iowa

Book Review Editor

DENNIS G. PAPPAS, MD .... Birmingham, Ala

Emeritus Board Members

WESLEY H. BRADLEY, MD .... Albany, NY
JACK D. CLEMIS, MD .... Chicago, Ill
SEYMOUR COHEN, MD .... Los Angeles, Calif
CHARLES W. CUMMINGS, MD .... Baltimore, Md
PATRICK J. DOYLE, MD .... Vancouver, Canada
ARNDT J. DUVALL III, MD .... Minneapolis, Minn
JOHN M. FREDRICKSON, MD .... St Louis, Mo
RICHARD R. GACEK, MD .... Syracuse, NY
JEROME C. GOLDSTEIN, MD .... Lake Worth, Fla
SIR DONALD F. N. HARRISON, MD PHD .... London, England
SAM E. KINNEY, MD .... Cleveland, Ohio
JOHN A. KIRCHNER, MD .... New Haven, Conn
DAVID J. LIM, MD .... Los Angeles, Calif
FRED H. LINTHICUM, JR, MD .... Los Angeles, Calif
MICHEL PORTMANN, MD .... Bordeaux, France
LORING W. PRATT, MD .... Waterville, Me
FRANK N. RITTER, MD .... Ann Arbor, Mich
ROBERT J. RUBEN, MD .... Bronx, NY
WILLIAM H. SAUNDERS, MD .... Columbus, Ohio
RUEDIGER THALMANN, MD .... St Louis, Mo
LESTER D. R. THOMPSON, MD .... Washington, DC
HARVEY M. TUCKER, MD .... Cleveland, Ohio
PAUL H. WARD, MD .... Los Angeles, Calif

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

— Founded in 1892 —

ANNALS
PUBLISHING CO.

EDITOR'S OFFICE

Brian F. McCabe, MD
Department of Otolaryngology –
Head & Neck Surgery
University Hospitals & Clinics
Iowa City, IA 52242
(319) 356-2310

BUSINESS OFFICE

Annals Publishing Company
4507 Laclede Avenue
St Louis, MO 63108
(314) 367-4987
Fax (314) 367-4988
E-mail: manager@Annals.com

STAFF

Managing Editor
Michol L. Sheridan

Business Manager
Kenneth A. Cooper, Jr

Chief Copyeditor
Edna Hayes Harrison

Copyeditor
Scott A. Bailey

Circulation Manager
Kevin R. Cooper

Editorial Assistant
Sarah G. Keith

ADVERTISING REPRESENTATIVES

— East & Midwest —
Cunningham Associates
180 Old Tappan Rd
Old Tappan, NJ 07675
(201) 767-4170
Fax (201) 767-8065
JPattis@cunnasso.com

— West —
Charles B. Healy
PO Box 5213
Palm Springs, CA 92263
(760) 320-0118

EDITORIAL CONTENT

The *ANNALS* publishes original manuscripts of clinical and research importance in otolaryngology – head and neck medicine and surgery, bronchoesophagology, laryngology, head and neck oncology and surgery, plastic and reconstructive surgery, pediatric otolaryngology, audiology, speech pathology, and related specialties. In-depth studies (supplements), papers of historical interest, reviews of computer software and applications in otolaryngology are also published, as well as imaging, pathology, and clinicopathology studies, book reviews, and letters to the editor. All papers and supplements are peer-reviewed. The *ANNALS* is the official journal of the American Laryngological Association and the American Broncho-Esophagological Association.

SUBSCRIPTIONS (INCLUDES ON-LINE SUBSCRIPTION)

2001 Annual Subscription Rates	*USA*	*International Surface Mail (All Regions)*
Institution*	$240.00	$265.00
Individual†	170.00	195.00
Resident/Student†	112.00	129.00

*Institutional (multiple-reader) subscription rates apply to public and private libraries, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and commercial and private institutions and organizations.

†Individual, resident, and student subscriptions must be in the individual's name and must be billed to and paid for by the individual.

Airmail rates are available upon request.

Visa, Mastercard, and American Express are accepted as payment.

WEB SITE

http://www.Annals.com

AVAILABILITY OF CD-ROM

CD-ROMs are available for each volume in March of the following year. The 1998 volume was the first on CD-ROM. Current subscribers can purchase a CD-ROM at $76.00 for an individual and $99.00 for an institution. Orders placed by non-subscribers are $175.00 for an individual and $245.00 for an institution.

CLAIMS & BACK ISSUES

Claims. Claims for missing issues must be received within 60 days of the month of publication for the United States and Canada, and 180 days for other countries. Single copy prices will be charged on missing issues claimed after that time.

Back Issues. A comprehensive inventory of back issues and supplements is available. Prices are furnished on request.

Change of Address. Address changes must be received 45 days before the effective date so that the subscription will be received without interruption.

MICROFILM

The *ANNALS* is available in microform from University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106.

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY (ISSN 0003-4894) is published monthly by Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108. Periodicals postage paid at St Louis, Mo. POSTMASTER: Send address changes to *ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY*, c/o Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108-2103.

COPYRIGHT© 2001, ANNALS PUBLISHING COMPANY

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

VOLUME 110 JANUARY 2001 NUMBER 1

CONTENTS

FROM THE AMERICAN LARYNGOLOGICAL ASSOCIATION

Mitomycin: Effects on Laryngeal and Tracheal Stenosis, Benefits, and Complications
Reza Rahbar, DMD, MD; Stanley M. Shapshay, MD; Gerald B. Healy, MD 1

Local Recurrences Following Transoral Laser Surgery for Early Glottic Carcinoma: Frequency, Management, and Outcome
Hans Edmund Eckel, MD 7

FROM THE AMERICAN BRONCHO-ESOPHAGOLOGICAL ASSOCIATION

Acquired Total (Grade 4) Subglottic Stenosis in Children
L. Mark Gustafson, MD; Benjamin E. J. Hartley, MD; Robin T. Cotton, MD 16

Effect of In Vitro Irradiation of Donor Larynges on Cyclosporine Requirements and Rejection Rates in Rat Laryngeal Transplantation
Steven W. Barthel, MD; Olivia Dan; Jonathan Myles, MD; Marshall Strome, MD 20

Effect of Mitomycin-C on Vocal Fold Healing in a Canine Model
C. Gaelyn Garrett, MD; Joseph Soto, MD; John Riddick;
Cheryl R. Billante, PhD; Lou Reinisch, PhD 25

INDEPENDENT CONTRIBUTIONS

Intraoperative Assessment of Stapes Movement
Alexander Huber, MD; Thomas Linder, MD; Mattia Ferrazzini, MS;
Stephan Schmid, MD; Norbert Dillier, PhD; Sandro Stoeckli, MD; Ugo Fisch, MD 31

Malignant Carotid Body Tumors: Report of Three Cases
Luis Pacheco-Ojeda, MD 36

Further Experience With Tracheojejunal Shunt Speech After Pharyngolaryngoesophagectomy
Minoru Kinishi, MD; Mutsuo Amatsu, MD; Shinya Tahara, MD 41

Performance Characteristics of Fluorescence Endoscope in Detection of Head and Neck Cancers
Boonchu Kulapaditharom, MD; Vipa Boonkitticharoen, PhD 45

Globosal Basal Cells Are Identified as Proliferating Cells in Mouse Olfactory Epithelium
Yasushi Ohta, MD; Keiichi Ichimura, MD 53

Effects of Transglottal Pressure on Fundamental Frequency of Phonation: Study With a Rubber Model
Hideyuki Kataoka, MD; Kazutomo Kitajima, MD; Shigehiro Owaki, MD 56

Endobronchial Tumors in Children: An Uncommon Clinical Entity
LT Kirby J. Scott, MC USN; John H. Greinwald, Jr, MD;
David Darrow, MD, DDS; Richard J. H. Smith, MD 63

Congenital Nasal Pyriform Aperture Stenosis: Diagnosis and Management of 20 Cases
Thierry Van Den Abbeele, MD, PhD; Jean-Michel Triglia, MD;
Martine François, MD; Philippe Narcy, MD 70

Immunohistochemical Analysis of Small Cell Carcinoma of the Head and Neck: A Report of Four Patients and a Review of Sixteen Patients in the Literature With Ectopic Hormone Production
Hiroyuki Mineta, MD; Katsutoshi Miura, MD; Satoru Takebayashi, MD;
Keisuke Araki, MD; Yoh Ueda, MD; Hidekazu Harada, MT; Kiyoshi Misawa, MD 76

Nitric Oxide Mediates Platelet Activating Factor–Induced Microvascular Leakage in Rat Airways
Sea-Yuong Jeon, MD; Eun-Ah Kim; Yong-Woon Ma, MD; Jin-Pyeong Kim, MD;
Tae-Gee Jung, MD; Eui-Gee Hwang, MD 83

Microbiology of Serous Otitis Media in Children: Correlation With Age and Length of Effusion
Itzhak Brook, MD, MSc; Paula Yocum, MSc; Kiran Shah, MD;
Bruce Feldman, MD; Stephen Epstein, MD 87

Volumetric and Dimensional Analysis of the Guinea Pig Inner Ear
Yusuke Shinomori, MD; Debra S. Spack; Diane D. Jones; Robert S. Kimura, PhD 91

IMAGING CASE STUDY OF THE MONTH

Ectopic Growth Hormone–Secreting Pituitary Adenoma in the Sphenoid Sinus
Dino Madonna, MD; Ady Kendler, MD, PhD; Ahmed M. S. Soliman, MD 99

MISCELLANEOUS

Letter to the Editor 102
Book Review 102
Information for Subscribers 2a
Index to Advertisers 10a

Courses on the Lateral Approaches to the Skull Base

(*In English*)

Course Director:
Mario Sanna, MD

This cadaver hands-on workshop provides the individual opportunity to participate in:

- Video presentation of techniques and clinical cases
- Didactic presentations
- Performing the surgical procedures on specially prepared bones and half-heads
- Discussions

For additional information, contact:

Secretary Gruppo Otologico
Via Emmanueli n.42
29100 Piacenza - Italy
telephone: 0523/754362
fax: 0523/453708
E-mail: Corsi.Sanna@otologic.it

A161

KAISER PERMANENTE
More people turn to us for good health.

DENVER, COLORADO OTOLARYNGOLOGY

The Colorado Permanente Medical Group is seeking a Board-eligible/certified otolaryngologist for a stimulating referral practice in a prepaid group setting. Salaried position with excellent benefits. Denver offers abundant four-season outdoor activities enhanced by many community amenities.

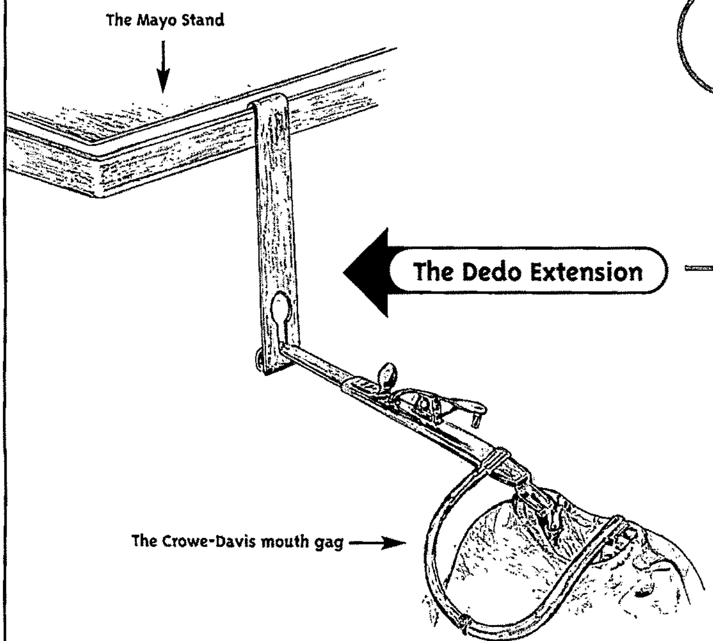
Contact:

Physician Recruitment
Colorado Permanente Medical Group
10350 E Dakota Ave
Denver, CO 80231
Phone: 303-344-7302
Fax: 303-344-7818

EOE,M/F,V/H

A187

Reach More Patients.



People come in different shapes and sizes. The same piece of equipment does not fit them all. Make oropharyngeal surgery easier with a simple extension. The CANT Corporation has created the Dedo Extension that fits between the Mayo Stand and the Crowe-Davis mouth gag so that it can be adjusted to fit larger patients. The Dedo Extension – a simple but effective solution.

Applicable Procedures

- T&A's
- Uvuloplasty
- Palatoplasty
- All oropharyngeal procedures using a Crowe-Davis mouth gag

cantcorpcan@begneaud.com 337·231·5830

CANT CORPORATION

INSTRUCTIONS TO AUTHORS

SUBMISSION

Send manuscripts to Editor Brian F. McCabe, MD, Department of Otolaryngology–Head and Neck Surgery, The University of Iowa Hospitals and Clinics, Iowa City, IA 52242. Original manuscripts dealing with clinical and scientific aspects of otolaryngology, bronchoesophagology, head and neck medicine and surgery, maxillofacial and plastic surgery, audiology, speech pathology, and related specialties are considered for publication. All materials submitted for publication undergo peer review. All manuscripts must be accompanied by a signed copyright transfer (see "Copyright," below). Include a self-addressed, stamped envelope for notification of receipt of manuscript.

Written permission from both senior author and publisher must be provided to the *Annals* in order to republish tables or illustrations copyrighted elsewhere. Submit this permission with the manuscript.

Papers are scheduled for publication in chronological order of acceptance. Manuscripts received in improper form require longer production time. Manuscripts are edited in accordance with the *AMA Manual of Style*, 9th edition (1998), and with the *Uniform Requirements for Manuscripts Submitted to Biomedical Journals* (*Ann Intern Med* 1988;108:258-65).

MANUSCRIPT PREPARATION

Limit papers to a size that will make up to no more than six printed pages, figuring three double-spaced typewritten pages of text to one typeset page; see the journal for estimating space required for references, illustrations, and tables. If a manuscript of greater length is accepted for publication by the Editor, all typeset pages over six are charged to the author at the publisher's cost of $150 per page. Submit an original and two copies of the manuscript, typed on white bond paper with margins of at least 25 mm (1 in), double-spaced throughout, including abstract, references, tables, and legends. Use a type size no smaller than 10 points, preferably 12. Begin each component on a new page in the following sequence: title page, abstract, text, acknowledgments, references, tables, and figure legends. Number pages consecutively in the upper right corner, beginning with the title page. Avoid the use of staples.

The author is responsible for all statements in the paper, as approved on the copyedited galley proofs. Alterations made by the author after the paper has been typeset are charged to the author.

Use standard abbreviations given in *Uniform Requirements*. Express all measurements in metric terms; if original measurements were made in another system, include these parenthetically. Plot audiograms according to ISO standards. Use generic names whenever possible.

Title page must include 1) a concise but informative title, worded to facilitate indexing; 2) an abbreviated form of the title to be used as a running head; 3) authors' full names (no more than seven authors are permitted) and no more than two academic degrees per author; 4) department(s) and institution(s) to which the work is attributed, with authors' present affiliations and addresses, if different, separately noted; 5) statement of grant or other support; 6) name and address of author to whom reprint requests should be sent; and 7) name, address, telephone and fax numbers, and, if possible, e-mail address of corresponding author. Manuscripts that report animal research performed in the United States must carry the following statement on the title page: "This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of __________ University (or institution)."

Abstracts contain no more than 150 words and state the goal of the study, primary procedures, main findings, and principal conclusions.

Key Words, chosen as far as possible from the National Library of Medicine *Medical Subject Headings*, are listed after the abstract.

References, double-spaced, are numbered consecutively in the order in which they are cited in the text. Primary references should be used whenever possible. The author is charged $1.50 for each reference over 30. Use the style of references given in *Uniform Requirements* or a current issue of the *Annals*. Include the names of all authors and the inclusive page numbers of an article. If a manuscript accepted but not yet published is included in the reference list, give the accepting journal's name, followed by "in press." Manuscripts still in review or not yet accepted formally are cited within the text as "unpublished observations." A reference to a personal communication is also placed in the text, accompanied by a date (year). Papers presented at scholarly meetings but not published are considered "unpublished observations." Papers published only in abstract form are listed as references with "[Abstract]" after the title.

Tables should be on separate sheets, numbered consecutively and headed by a concise title. Put explanatory matter in footnotes. Tables are adjuncts to the text and should not repeat material presented therein. The cost of typesetting tables is billed to the author.

Illustrations must be submitted in three complete sets, unmounted. Only professional-quality glossy photographs and black-and-white line drawings are acceptable. Computer-generated graphs are acceptable only if they are clear and will reproduce well. Letters, numbers, and symbols should be clear and of uniform size throughout, large enough that each item is legible when reduced for publication. Put legends (detailed explanations) to the photographs on a separate page in the manuscript. Affix a label to the reverse side of each illustration indicating figure number, first author's name, and top of the figure. For radiographs, photomicrographs, CT scans, etc, give indications for maximum cropping and labeling on an overlay or photocopy. Cite each figure in the text in consecutive order. Written permission from identifiable subjects is required. The cost of preparing illustrations for publication (sizing to fit journal, and preparation of negatives for printing) is charged to the author without exception. Color illustrations are accepted; cost estimates for color separations and printing are provided on an individual basis. Illustrations should enhance, not repeat, material presented in the manuscript, and should be kept to a minimum.

REVIEWS OF SOFTWARE AND COMPUTER APPLICATIONS

Programs submitted for review must be compatible with an IBM or Macintosh computer, and the author must specify hardware and system requirements. The following operating systems will be supported: MS-DOS; MS-DOS with Windows; and MAC-OS. Submit the software on either a 3.5" or 5.25" disk. If the program is a template, submit also a runtime version of the source program. Software considered for review includes educational software, artificial intelligence software to aid in the diagnosis of otolaryngic disorders, software for logging operations, software to aid in research, and software that addresses specific problems in otolaryngology. Also, manuscripts reviewing software and computer applications relating directly to otolaryngology are considered for publication. See *Ann Otol Rhinol Laryngol* 1991; 100:876 for specific instructions.

SUPPLEMENTS

A manuscript too long for inclusion in the *Annals* (over 12 typeset pages) may be published as a supplement if approved by the Editor. All costs are borne by the author and estimates are provided upon request. Supplements have the advantages of separate identification and rapid publication, but undergo the same critical review as journal articles.

REPRINTS

Rates are quoted when galley proofs are sent to the author. Orders are signed by the author and returned with the proofs.

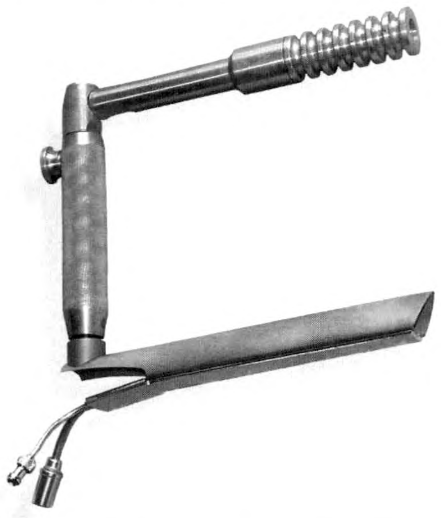
COPYRIGHT

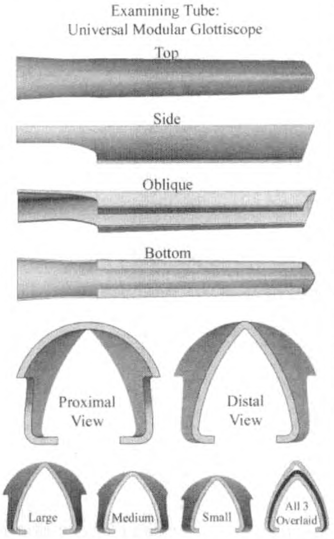
The following statements should accompany the manuscript, signed by all authors (only original signatures are acceptable): 1) "This paper has not been published or submitted for publication elsewhere." 2) [*For papers with more than one author*] "I have contributed substantively to the development of the content of this paper and have agreed to have my name listed as an author in the final, revised version." 3) "In consideration of the *Annals of Otology, Rhinology & Laryngology* taking action in reviewing and editing my (our) submission, I hereby transfer, assign, or otherwise convey all copyright ownership to Annals Publishing Company in the event such work is published in the *Annals of Otology, Rhinology & Laryngology*."

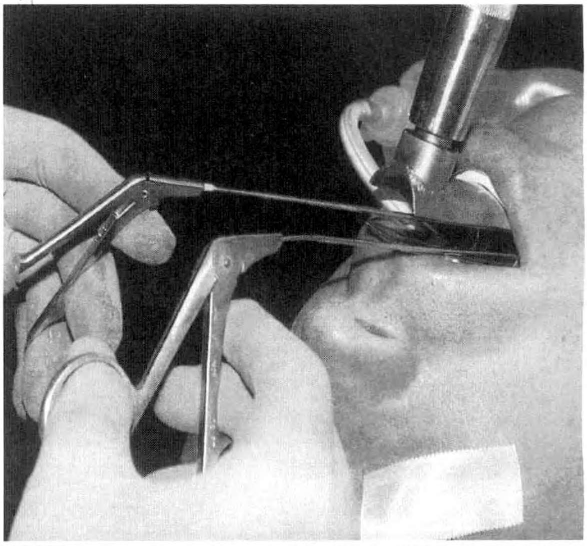
The Zeitels Universal Modular (UM) Glottiscope System

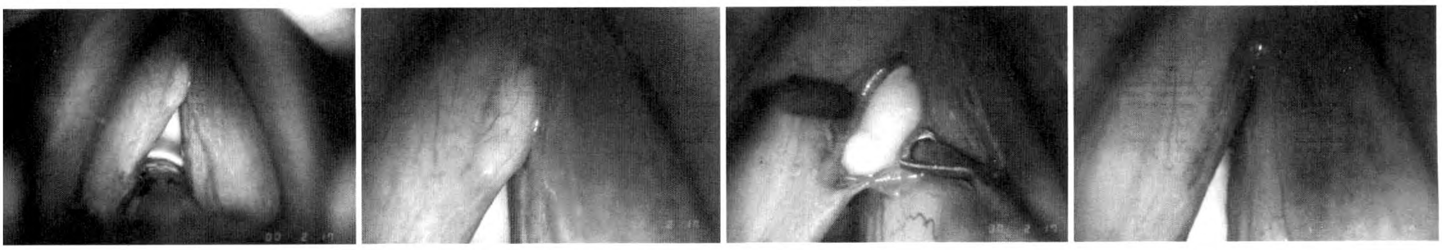
The Evolution of a Century of Design

The recently patented UM glottiscope system was conceived from the study of a century of direct laryngoscope designs to incorporate the most valuable innovations into a uniquely amalgamated instrument. This glottiscope system provides the clinician with a versatile laryngoscope that optimally exposes vocal folds for diagnosis and nstrumental manipulation, regardless of the diversity of human anatomical factors: age, gender, and pathology.









Design Features (US patent 5,893,830; international patents pending)

- The distal lumen of the UM glottiscope is a triangular lancet-arch configuration that distracts the false vocal cords and conforms to the anterior glottal commissure.
- Unlike virtually all microscope-compatible tubular laryngoscopes, which widen the proximal aperture to facilitate angulation of hand instruments, the UM glottiscope has bilateral proximal slots that dramatically improve the positioning of instrumentation.
- The UM glottiscope is supplied with a variety of examining tubes which accommodate to the spectrum of human size and shape, irrespective of gender, age, or disease, and that attach to a single universal handle.
- The universal, ergodynamically designed titanium handle can be joined with a suspension gallows as well as American and European chest-support holders.
- The detachable base-plate is ideally suited for difficult intubations; lighting and suction cannulae are contained within the base-plate.

ENDOCRAFT® LLC

Evolutionary Designs for Laryngeal Instrumentation

WEB SITE—http://www.endocraft.com

Phone: (401) 454-7267(SCOP) **Fax:** (401) 331-2742 **E-mail:** endocraft1@aol.com

Ann Otol Rhinol Laryngol 110:2001

MITOMYCIN: EFFECTS ON LARYNGEAL AND TRACHEAL STENOSIS, BENEFITS, AND COMPLICATIONS

REZA RAHBAR, DMD, MD

STANLEY M. SHAPSHAY, MD GERALD B. HEALY, MD

BOSTON, MASSACHUSETTS

The management of laryngeal and tracheal stenosis continues to challenge us, despite advances in surgical techniques and better understanding of the wound healing process. Injury to the airway mucosa is the inciting event, regardless of the cause of the stenosis. Mitomycin-C is an antineoplastic antibiotic that acts as an alkylating agent by inhibiting DNA and protein synthesis. It can inhibit cell division, protein synthesis, and fibroblast proliferation. Topical application of mitomycin-C (0.4 mg/mL) was used as an adjuvant treatment in the endoscopic laser management of laryngeal and tracheal stenosis in 15 patients. Fourteen patients (93%) have shown improvement of their airway and resolution of their preoperative symptoms. After a mean follow-up of 18 months, no complication was noted with regard to the application of mitomycin-C. This study gives promising findings on the efficacy and safety of mitomycin-C as an adjuvant treatment in the management of selected cases of laryngeal and tracheal stenosis.

KEY WORDS — endoscopic surgery, laryngeal stenosis, laser surgery, mitomycin-C, subglottic stenosis, tracheal stenosis.

INTRODUCTION

Treatment of laryngeal and tracheal stenosis remains one of the most difficult and perplexing problems in the area of head and neck surgery. Scar formation and restenosis remains the main cause of failure in the surgical management of airway stenosis. Modulation of the wound healing process to prevent excessive scar formation can play a major role in improving the success rate and decreasing the need for further surgery.

Mitomycin-C is an antiproliferative agent that can inhibit fibroblast proliferation and activity. It has been successfully used as an adjuvant treatment in a number of ophthalmologic procedures to decrease scar formation and restenosis.

A multicenter prospective clinical trial was undertaken in an effort to evaluate the efficacy and safety of the topical application of mitomycin-C in the prevention and treatment of airway stenosis. Mitomycin-C was used as an adjuvant treatment in the endoscopic laser management of laryngeal or tracheal stenosis in 15 patients. The preliminary results of some of these patients have been reported.[1]

MATERIALS AND METHODS

Fifteen patients underwent endoscopic laser management of laryngeal or tracheal stenosis and the topical application of mitomycin-C at New England Medical Center and The Children's Hospital, Boston, between January 1998 and December 1999. There were 8 male patients and 7 female patients. The patients' ages ranged from 2 to 78 years (mean, 32 years). The patients presented with preoperative symptoms of dyspnea interfering with routine daily activity, poor voice quality and vocal fatigue, and/or the inability to cap their tracheotomy tube. Included were 3 cases of glottic stenosis, 10 cases of subglottic stenosis, and 2 cases of tracheal stenosis (see Table).

Glottic Stenosis. One patient (No. 1) presented with a congenital anterior glottic web. Two patients (Nos. 2 and 3) were referred with posterior glottic stenosis and arytenoid fixation: 1 patient had undergone a tracheotomy and laryngofissure for repair of a blunt laryngeal trauma, and the other had undergone a tracheotomy and partial arytenoidectomy for bilateral vocal cord paralysis caused by a brain stem infarct. Only patient 3 was tracheotomy-dependent at the time of our evaluation, with an inability to cap the tracheotomy tube and poor voice quality.

Subglottic Stenosis. Three patients (Nos. 5, 11, and 13) presented with subglottic stenosis due to prolonged intubation because of cardiac surgery, pneumonia, and thalamic infarct, respectively. One patient (No. 4) presented with restenosis after a laryngotracheal reconstruction for a congenital subglottic stenosis (grade IV). No cause could be identified in

---

From the Department of Otolaryngology and Communication Disorders, The Children's Hospital (Rahbar, Healy), the Department of Otology and Laryngology, Harvard Medical School (Rahbar, Healy), the Department of Otolaryngology–Head and Neck Surgery, New England Medical Center (Shapshay), and Tufts University School of Medicine (Shapshay), Boston, Massachusetts.

Presented at the meeting of the American Laryngological Association, Orlando, Florida, May 13-14, 2000.

CORRESPONDENCE — Reza Rahbar, DMD, MD, Dept of Otolaryngology and Communication Disorders, The Children's Hospital, 300 Longwood Ave, Boston, MA 02115.

PATIENT DATA

Pt No.	*Sex*	*Age (y)*	*Location of Stenosis*	*Etiology*	*Tracheotomy-Dependent*	*No. of Procedures*	*Initial Airway Stenosis*	*Thickness (mm)*	*Current Airway Stenosis*	*Initial Symptoms*	*Current Status*	*Follow-up (mo)*
1	F	5	Glottic	Congenital web	No	2	60%	4	20%	B	F	12
2	M	14	Glottic	Laryngeal trauma	No	1	4-mm opening		7-mm opening	A, B	F	23
3	M	78	Glottic	Bilateral vocal cord paralysis	Yes	3	2-mm opening		5-mm opening	B, C	D, E	25
4	M	14	Subglottic*	Congenital	Yes	1	80%	4	20%	B, C	D, E	6
5	M	17	Subglottic	Prolonged intubation	No	1	80%	5	30%	A, B	F	13
6	F	33	Subglottic*	Idiopathic subglottic stenosis	No	1	80%	15	20%	A, B	F	25
7	F	38	Subglottic	Idiopathic subglottic stenosis	No	1	50%	12	20%	A, B	F	27
8	F	40	Subglottic*	Idiopathic subglottic stenosis	No	1	80%	4	20%	A, B	F	15
9	F	42	Subglottic*	Idiopathic subglottic stenosis	No	1	50%	7	10%	A, B	F	15
10	F	45	Subglottic*	Idiopathic subglottic stenosis	No	2	70%	10	10%	A, B	F	20
11	M	48	Subglottic	Prolonged intubation	Yes	2	95%	4	40%	A, B, C	D, E	19
12	M	53	Subglottic	Wegener's granulomatosis	No	1	50%	5	10%	A	F	15
13	M	56	Subglottic	Prolonged intubation	No	2	70%	5	30%	A, B	F	29
14	M	2	Tracheal	Prolonged intubation	No	1	70%	5	60%	Prolonged intubation	Tracheotomy	15
15	F	5	Tracheal	Congenital stenosis	No	2	70%	3	10%	A, B	F	13

A — dyspnea on routine daily activity; B — poor voice quality and vocal fatigue; C — inability to cap tracheotomy tube; D — improved voice quality; E — ability to cap tracheotomy tube during day; F — resolution of preoperative symptoms.

*Circumferential scarring.

5 cases (patients 6 through 10), which were classified as idiopathic subglottic stenosis. Wegener's granulomatosis was the cause of subglottic stenosis in 1 patient (No. 12). Only 2 patients from this group (Nos. 4 and 11) were tracheotomy-dependent, with an inability to cap the tracheotomy tube and poor voice quality.

Tracheal Stenosis. One patient (No. 14), with a history of trisomy 21, presented with tracheal stenosis after prolonged intubation because of cardiac and pulmonary status. A second patient (No. 15) presented with tracheal stenosis after laryngotracheal reconstruction for a congenital subglottic and tracheal stenosis.

All patients underwent endoscopic examination and treatment under inhalational general anesthesia provided via a tracheotomy tube, when present, or intravenous anesthesia and Venturi jet ventilation. The airway size and length of stenosis were measured with an open tube ventilating bronchoscope or an endotracheal tube through a laryngoscope. Surgical exposure was obtained by placing the tip of a laryngoscope (Dedo, Healy-Jako, or anterior commissure scope) through the vocal cords for subglottic and tracheal stenosis. The laryngoscope was suspended from a Boston University suspension apparatus, and an operating microscope was used at 16× magnification for all cases. The CO_2 laser at a power setting of 4 to 6 W (time, 0.1 to 0.3 second), in a

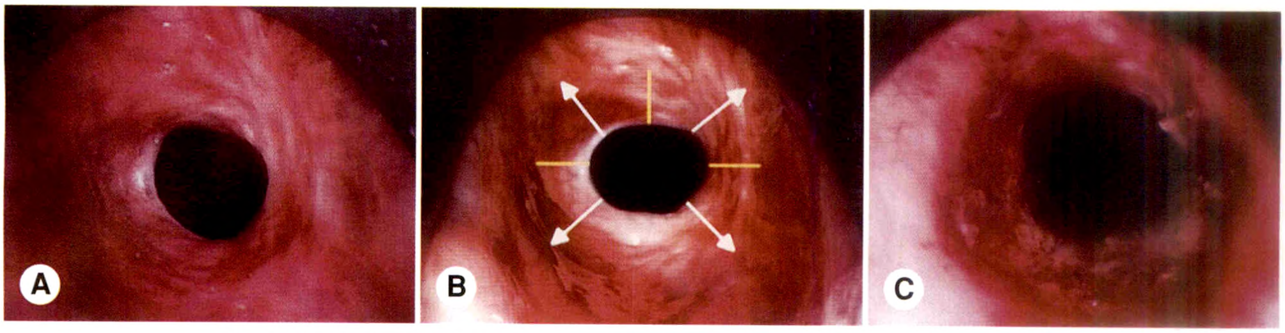


Fig 1. Subglottic stenosis. A) Preoperative. B) Radial laser incision. C) Postoperative.

superpulsed mode (0.25- to 0.3-mm spot size) was used to make radial incisions through the subglottic or tracheal stenosis (Fig 1). Ventilating open tube bronchoscopes were then used for dilation of the stenotic segment. The radial incisions in subglottic or tracheal stenotic areas served to guide the dilating forces in a controlled manner to preserve viable epithelium within the incision.[2] A similar laser setting was used for excision of glottic stenosis (Fig 2). Thereafter, a 10-mm cottonoid sponge was soaked with mitomycin-C 0.4 mg/mL and topically applied to the surgical site for 4 minutes. Then the area was irrigated with 20 to 30 mL of normal saline solution. All patients were observed for a minimum of 24 hours after the procedure. Success was defined as an improvement in the size of the airway and elimination of the preoperative symptoms.

RESULTS

A total of 22 procedures were performed in 15 patients (average, 1.5 procedures): 9 patients had 1 procedure each, 5 patients had 2 procedures each, and 1 patient had 3 procedures. The follow-up ranged from 6 to 29 months, with an average of 18 months. Open tube bronchoscopes and/or endotracheal tubes were used for the measurement of the postoperative length and degree of stenosis under general anesthesia, or the measurements were made with fiberoptic laryngoscopy and still photographs taken by means of a 70° rigid telescope under topical anesthesia. Fourteen patients (93%) showed an improvement in the degree of airway stenosis and resolution of their preoperative symptoms (see Table). The postoperative length of the subglottic or tracheal stenosis could only be measured in patients who underwent general anesthesia because of the need for further surgery. There was no significant improvement noted in the length of the stenosis.

Glottic Stenosis. A total of 6 procedures were performed in 3 patients. Before operation, 1 patient demonstrated a 60% stenosis, and 2 patients had posterior airway openings of 4 and 2 mm, respectively, compared to a postoperative stenosis of 20% and airway openings of 7 and 5 mm, respectively. Two patients are completely asymptomatic with regard to their preoperative symptoms. One patient was tracheotomy-dependent, was unable to cap the tracheotomy tube, and had poor voice quality. Currently, the tracheotomy tube is capped during the day without dyspnea on exertion, and the patient has good voice quality.

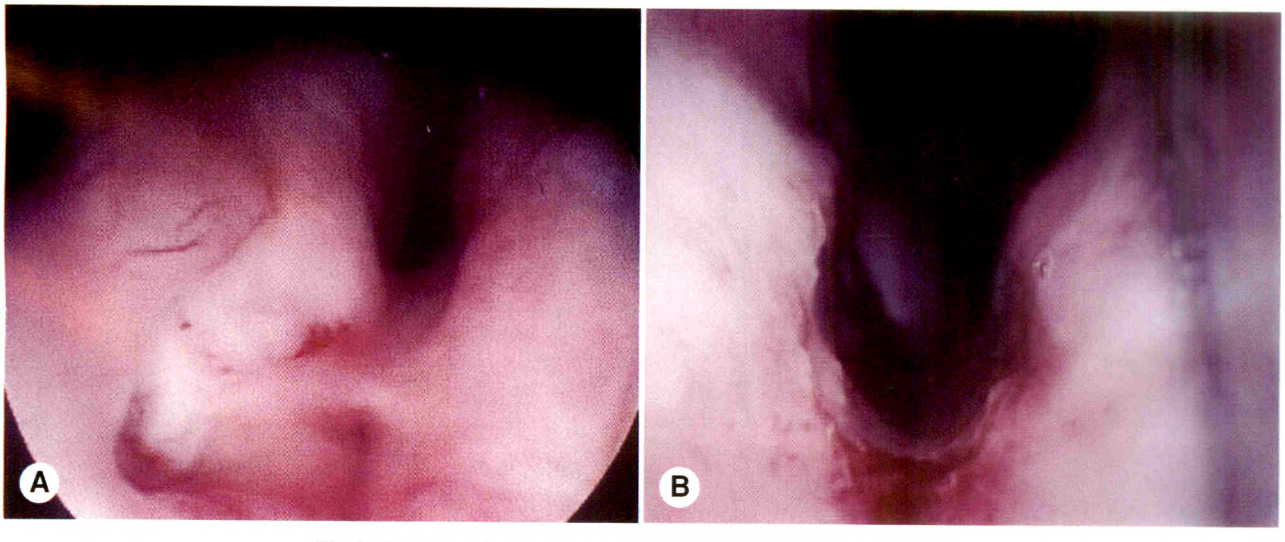


Fig 2. Posterior glottic stenosis. A) Preoperative. B) Postoperative.

The patient has not been decannulated because of his general medical condition following a brain stem infarct.

Subglottic Stenosis. A total of 13 procedures were performed in 10 patients. The preoperative stenosis ranged from 50% to 95% (mean, 71%), and the postoperative stenosis ranged from 10% to 40% (mean, 21%). The length of the stenosis ranged from 4 to 15 mm (average, 7 mm). Three patients had a length of stenosis equal to or greater than 1 cm. Before operation, 9 patients had moderate dyspnea with routine daily activity; currently, they are all free of symptoms. Two patients were tracheotomy-dependent before the procedure, with an inability to cap the tracheotomy tube and poor voice quality. Currently, both patients tolerate capping the tracheotomy tubes during the day and have improved voice quality. One patient (No. 11) has not been decannulated because of his general medical condition (diabetes and recurrent pneumonia); the second patient (No. 4) is scheduled for decannulation in the near future.

Tracheal Stenosis. A total of 3 procedures were performed in 2 patients. The preoperative degree of stenosis was 70% in both patients; the stenoses were 3 and 5 mm long, respectively. One patient (No. 15) is asymptomatic with 10% airway obstruction. The second patient (No. 14), whose history is significant for trisomy 21, tetralogy of Fallot, and pulmonary arteriovenous malformation, presented with restenosis and underwent tracheotomy because of worsening of his pulmonary and cardiac status.

DISCUSSION

The management of laryngeal and tracheal stenosis remains difficult, requiring accurate diagnosis and a proper treatment plan. The goal of any treatment is the restoration of an adequate airway, the provision of a competent larynx for protection against aspiration, and the achievement of a satisfactory voice. The first decision is whether an open surgical technique is necessary, or whether an endoscopic approach is adequate. Factors such as 1) age, 2) general medical condition, 3) cause, location, degree, length, and consistency of stenosis, and 4) previous treatment should be considered. There is still controversy with regard to the optimum approach in the treatment of laryngeal and tracheal stenosis.

Endoscopic management of laryngeal and tracheal lesions has undergone tremendous advancement in the past 2 decades. The development of microlaryngoscopy and adaptation of the binocular operating microscope, the introduction of endolaryngeal CO2 laser surgery, the coupling of an endoscopic adapter to a ventilating bronchoscope, and the development of the microspot manipulator allow microprecision and hemostatic ability, which were lacking with the standard instrumentation of conventional endoscopic techniques.[3-6]

Multiple endoscopic techniques have been described: microcauterization, dilation with local and systemic steroids, and cryosurgery have all had some success.[7,8] Strong and Jako[6] first published their experience with endoscopic CO2 laser management of laryngeal stenosis in 1972. The CO2 laser appears to be the most effective means for treating mild to moderate stenosis of the larynx and trachea endoscopically.[9-12]

With the advancements in endoscopic surgical technique and a better understanding of the wound healing process, and even with the introduction of mucosa-preserving laser surgery such as the microtrapdoor flap technique (Dedo and Sooy[13]) and radial incision and dilation (Shapshay et al[2]), there is still a high degree of scar formation and restenosis. A review of the literature reveals a wide range of success for endoscopic management of laryngeal and tracheal stenosis: Strong et al,[14] 50% success; Andrews and Horowitz,[15] 66% success; Simpson et al,[16] 44% success; Carruth,[17] 50% success; Shapshay and Simpson,[5] 40% success; and Ossoff et al,[18] 57% success. Although there are fundamental differences between these studies (definition of success; location, severity, and type of stenosis; length of follow-up), restenosis and scar formation remains the primary cause of failure.

Regardless of the surgical techniques, there is further injury to the airway mucosa that leads to fibroblast proliferation and collagen formation, which are the key to scar formation. Modulation of the wound healing response to prevent excessive scar formation can play a major role in increasing the success of surgical treatment and reducing the need for further surgery. In the past decade, the role of growth factors and fibrogenic peptides in the regulation and modulation of wound healing leading to fibrosis and scar formation has been reported.[19-21] Pharmacological agents such as 5-fluorouracil and β-aminopropionitrile (βAPN) have been shown to inhibit the development of collagen cross-linking, thus minimizing scar formation.[22,23]

Kunitomo and Mori[24] presented the first clinical use of mitomycin-C in the prevention and treatment of scar formation in pterygium surgery. Later, Chen,[25] Palmer,[26] and Bergstrom et al[27] reported its use for prevention of restenosis in glaucoma surgery.

Mitomycin-C is an antibiotic produced by *Streptomyces caespitosus.* It is referred to as mitomycin-C to differentiate it from mitomycins A and B. It has both antineoplastic and antiproliferative properties. Its antineoplastic activity is similar to that of the alkylating agents, causing cross-linking of DNA and inhibiting RNA and protein synthesis. Mitomycin-C also acts as an antiproliferative agent that can inhibit fibroblast activity and suppress fibrosis and scar formation.

The exact mechanism by which mitomycin-C exerts an antifibroblast activity is unknown. There is evidence to suggest that the reduction of fibroblast activity may be mediated by apoptosis, which is a gene-directed process causing cell death. Apoptotic cells display a characteristic morphology that includes condensation of the nucleus and cytoplasm, nuclear fragmentation, and cytoplasmic blebbing with an intact cell membrane.[28] Lee et al[29] showed that mitomycin has antifibroblastic properties in vivo. Khaw et al[30] showed that in vitro, a single application of mitomycin could inhibit fibroblast proliferation. Mitomycin has been successfully used for prevention of stenosis in glaucoma surgery, dacryocystorhinostomy, optic nerve sheath fenestration, and pterygium recurrence.[31-35] The fibroblast population and collagen formation are substantially increased during the wound healing response that follows a mucosal insult such as a surgical procedure. The rationale of the use of mitomycin-C is to inhibit fibroblast proliferation during the postoperative phase without damaging the mucosal and epithelial growth.[36]

Historically, endoscopic management of patients with circumferential scarring, a vertical scar greater than 1 cm, posterior scarring with arytenoid fixation, and loss of cartilaginous support had a high failure rate.[16] We have presented 15 patients with laryngeal and tracheal stenosis. A group of these patients have characteristics of airway stenosis that falls into the category of high failure rate based on historical experience: 5 patients with circumferential stenosis of the subglottic area; 2 patients with posterior glottic stenosis and arytenoid fixation; and 3 patients with length of subglottic stenosis greater or equal to 1 cm. The endoscopic management of airway stenosis has produced a success rate of 40% to 68%, as reported by experienced investigators, with most results being closer to 50%. We have defined success as clinical improvement of the degree of stenosis and the resolution of preoperative symptoms. We have shown improvement of the size of the airway and resolution of preoperative symptoms in 14 patients (93% success rate), without any complications, with a mean follow-up of 18 months. Three of our patients were tracheotomy-dependent at the time of our evaluation. They have shown improvement of their airway, allowing capping of their tracheotomy tube during the day, and improvement of their voice quality. Two of these patients have not been decannulated because of their general medical condition, and 1 is scheduled for decannulation in the near future.

Our results demonstrate that the topical application of mitomycin-C can be beneficial in the modulation of wound healing and in decreasing scar formation in the treatment of airway stenosis. Our study is the first to describe the use of mitomycin-C as an adjuvant treatment in the endoscopic management of laryngeal and tracheal stenosis in both pediatric and adult populations. Further research and randomized prospective clinical trials are needed to determine the most effective concentration and time of exposure of mitomycin-C, and the difference in efficacy and safety between single and multiple applications.

CONCLUSION

Treatment of laryngeal and tracheal stenosis remains a therapeutic challenge. Different surgical techniques have been proposed, and no single approach has proven satisfactory for all cases. With the advances in the surgical techniques and the recognition of the importance of mucosal preservation, the potential for endoscopic surgical techniques has expanded. Restenosis and scar formation remains the main cause of failure in these cases. This study reveals promising results in terms of the efficacy and safety of mitomycin-C as an adjuvant method for the management of laryngeal and tracheal stenosis.

REFERENCES

1. Rahbar R, Valdez TA, Shapshay SM. Preliminary results of intraoperative mitomycin C in the treatment and prevention of glottic and subglottic stenosis. J Voice 2000;14:282-6.

2. Shapshay SM, Beamis JF Jr, Hybels RL, Bohigian RK. Endoscopic treatment of subglottic and tracheal stenosis by radial laser incision and dilation. Ann Otol Rhinol Laryngol 1987; 96:661-4.

3. Kleinsasser O. Microlaryngoscopy and endolaryngeal microscopy. Philadelphia, Pa: WB Saunders, 1968.

4. Polanyi TG, Bredemeier HC, Davis TW. A CO2 laser for surgical research. Med Biol Eng Comput 1970;8:541-8.

5. Shapshay SM, Simpson GT. Lasers in bronchology. Otolaryngol Clin North Am 1983;16:879-86.

6. Strong MS, Jako GJ. Laser surgery in the larynx. Early clinical experience with continuous CO2 laser. Ann Otol Rhinol Laryngol 1972;81:791-8.

7. Strome M. Cryosurgery: the effect on the canine endolaryngeal structures. Laryngoscope 1971;81:1057-65.

8. Friedman EM, Healy GB, McGill TJ. Carbon dioxide laser management of subglottic and tracheal stenosis. Otolar-

yngol Clin North Am 1983;16:871-7.

9. Strong MS, Healy GB, Vaughan CW, Fried MP, Shapshay SM. Endoscopic management of laryngeal stenosis. Otolaryngol Clin North Am 1979;12:797-805.

10. Healy GB. An experimental model for the endoscopic correction of subglottic stenosis with clinical applications. Laryngoscope 1982;92:1103-15.

11. Shapshay SM, Beamis JF Jr, Dumon J-F. Total cervical tracheal stenosis: treatment by laser, dilation, and stenting. Ann Otol Rhinol Laryngol 1989;98:890-5.

12. Strong MS, Jako GJ, Polanyi TG, Wallace RA. Laser surgery in the aerodigestive tract. Am J Surg 1973;126:529-33.

13. Dedo HH, Sooy CD. Endoscopic laser repair of posterior glottic, subglottic, and tracheal stenosis by division or micro-trapdoor flap. Laryngoscope 1984;94:445-50.

14. Strong MS, Vaughan CW, Polanyi G, Wallace R. Bronchoscopic carbon dioxide laser surgery. Ann Otol Rhinol Laryngol 1974;83:769-76.

15. Andrews AA, Horowitz SL. Bronchoscopic CO2 laser surgery. Lasers Surg Med 1980;1:35-9.

16. Simpson GT, Strong MS, Healy GB, Shapshay SM, Vaughan CW. Predictive factors of success or failure in the endoscopic management of laryngeal and tracheal stenosis. Ann Otol Rhinol Laryngol 1982;91:384-8.

17 Carruth AS. The role of lasers in otolaryngology. World J Surg 1983;7:719-22.

18. Ossoff RH, Tucker GF, Duncavage JA, Toohill RJ. Efficacy of bronchoscopic carbon dioxide laser surgery for benign strictures of the trachea. Laryngoscope 1985;95:1220-3.

19. Sera VR, Hall K, Low H, eds. Growth factors: from genes to clinical application. New York, NY: Raven Press, 1990.

20. Border WA, Ruoslahti E. Transforming growth factor–β in disease: the dark side of tissue repair. J Clin Invest 1992;90: 1-7.

21. Crouch E. Pathobiology of pulmonary fibrosis. Am J Physiol 1990;259:159-84.

22. Doolin EJ, Strande LF, Tsuno K, Santos MC. Pharmacologic inhibition of collagen in an experimental model of subglottic stenosis. Ann Otol Rhinol Laryngol 1998;107:275-9.

23. Ingrams DR, Ashton P, Dhingra J, Shah R, Shapshay SM. Slow-release 5-fluorouracil and triamcinolone reduces subglottic stenosis in a rabbit model. Ann Otol Rhinol Laryngol 2000; 109:422-4.

24. Kunitomo N, Mori S. Studies on the pterygium. Part IV. A treatment of the pterygium by mitomycin-C instillation. Acta Soc Ophthalmol Jpn 1963;67:601-7.

25. Chen C. Enhanced intraocular pressure controlling effectiveness of trabeculectomy by local application of mitomycin-C. Trans Asia-Pacific Acad Ophthalmol 1983;9:172-7.

26. Palmer S. Mitomycin as adjunct chemotherapy with trabeculectomy. Ophthalmology 1991;98:317-21.

27. Bergstrom TJ, Wilkinson WS, Skuta GL, Watnick RL, Elner VM. The effects of subconjunctival mitomycin-C on glaucoma filtration surgery in rabbits. Arch Ophthalmol 1991;109: 1725-30.

28. Desmouliere A, Redard M, Darby I, Gabbiani G. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. Am J Pathol 1995;146:56-66.

29. Lee DA, Lee TC, Cortes AE, Kitada S. Effects of mithramycin, mitomycin, daunorubicin, and bleomycin on human subconjunctival fibroblast attachment and proliferation. Invest Ophthalmol Vis Sci 1990;31:2136-44.

30 Khaw PT, Doyle JW, Sherwood MB, Grierson I, Schultz GS, McGorray S. Prolonged localized tissue effects from 5-minute exposures to fluorouracil and mitomycin C. Arch Ophthalmol 1993;111:263-7.

31. Palmer SS. Mitomycin as adjunct chemotherapy with trabeculectomy. Ophthalmology 1991;98:317-21.

32. Urban RC Jr, Kaufman LM. Mitomycin in the treatment of hypertrophic conjunctival scars after strabismus surgery. J Pediatr Ophthalmol Strabismus 1994;31:96-8.

33. Kao SCS, Liao CL, Tseng JHS, Chen MS, Hou PK. Dacryocystorhinostomy with intraoperative mitomycin. Ophthalmology 1997;104:86-91.

34. Rubinfeld RS, Stein RM. Topical mitomycin-C for pterygia: is single application appropriate? Ophthalmic Surg Lasers 1997;28:662-9.

35. Schipper I, Suppelt C, Gebbers J. Mitomycin-C reduces scar formation after excimer laser (193) photorefractive keratectomy in rabbits. Eye 1997;11:649-55.

36. Lee DA. Antifibrosis agent in glaucoma surgery. Invest Ophthalmol Vis Sci 1994,35:3789-91.

Ann Otol Rhinol Laryngol 110:2001

LOCAL RECURRENCES FOLLOWING TRANSORAL LASER SURGERY FOR EARLY GLOTTIC CARCINOMA: FREQUENCY, MANAGEMENT, AND OUTCOME

HANS EDMUND ECKEL, MD
COLOGNE, GERMANY

Although transoral laser surgery (TLS) for the treatment of early stage glottic carcinoma is now widely used, the patterns of local recurrences, related re-treatment methods, and results have not been documented comprehensively. Two hundred fifty-two patients with glottic carcinoma stage I or II were treated for cure with TLS alone and followed up for 24 to 139 months (mean, 62 months). Their charts were retrospectively reviewed to identify local recurrence patterns. Thirty-five patients (13.9%) presented with local recurrences or second laryngeal primaries 4 to 84 months (mean, 23 months) after initial treatment. Of the 161 patients classified T1N0M0, 21 (13.0%) suffered local recurrences, and in the 91 classified T2N0M0, 14 (15.4%) tumors recurred. If tumors recurring more than 60 months after initial treatment are considered second primary tumors rather than recurrences, then only 18 (11.2%) of 161 patients classified T1N0M0 would have had a recurrence. However, the difference in local control between patients with stage I versus stage II disease would still not be significant ($p = .41$). Of the 35 patients with local recurrences, 16 (45%) were managed with total laryngectomy, 10 (28.6%) with further TLS, 4 (11.4%) with partial laryngectomy, and 2 (5.7%) with radiotherapy, and 3 (8.6%) had no curative treatment. Accordingly, 16 patients (45.7%) with local treatment failure could be treated with further organ-sparing treatment methods. The actuarial overall survival, disease-specific survival, and organ preservation rates 5 years after the diagnosis of recurrent disease were 43.6%, 74.6%, and 33.7%. Transoral laser surgery leads to local control rates that are comparable to those found after radiotherapy for lesions classified T1 and leads to slightly better control rates for lesions classified T2, but the results are inferior to those achieved with conventional partial laryngectomy. However, if local recurrence occurs, then more re-treatment options are available after TLS as compared to initial radiotherapy or open surgery.

KEY WORDS — early glottic carcinoma, laryngeal neoplasm surgery, laryngectomy, laser surgery recurrence.

INTRODUCTION

For early (T1N0M0 and T2N0M0) squamous cell glottic carcinoma, excellent local control and survival rates have been reported for radiotherapy and for partial laryngectomy.[1-5] Besides these well-established oncological approaches, transoral laser surgery (TLS) has emerged as a major therapeutic alternative for early glottic and supraglottic tumors during the past 20 years.[6-11] It has some well-known advantages over open surgical procedures. Tracheotomy, which has been found to provide substantial negative impact on postoperative patient adjustment and quality of life after open partial surgery,[12] is usually not required.[13] The perioperative morbidity rate is low, and hospitalization is usually short.[14] Deglutition is usually not disturbed, whereas extended partial laryngectomy frequently leads to more-or-less prolonged aspiration.[13,15] As compared to radiotherapy, TLS is less time-consuming for the patient and is obviously more cost-effective.[13,16] A steadily growing body of literature, both from the United States and from Europe, confirms that TLS can be used to successfully treat patients with early-stage (stages I and II according to UICC and AJCC) glottic carcinoma.[17-20] In a recent survey on the patterns of care for cancer of the larynx in the United States, laser surgery accounted for 43.7% of all surgical interventions and for 24.8% of all treatments reported during the period 1990 to 1992, whereas it was used for only 34.2% of all surgical interventions and for 20.7% of all treatments reported during the period 1980 to 1985.[21] These data confirm the increasing importance of TLS for the treatment of larynx carcinoma. However, the patterns of local, regional, and distant recurrences, related re-treatments, and results of second-line treatment have not been documented comprehensively to date.[20,22,23]

This study seeks to evaluate the patterns of local recurrences, related re-treatments, and results for a consecutive cohort of patients treated with TLS and for the subset of patients with subsequent local treatment failures or second primaries in the larynx. The main outcome measures are the rates of overall survival, disease-specific survival, local control with initial therapy, and organ preservation.

PATIENTS AND METHODS

Patients and Treatment Selection. Six hundred seventy patients with previously untreated squamous cell carcinoma of the larynx were seen at the Department of Otorhinolaryngology, University of Cologne, Ger-

---

From the Department of Otorhinolaryngology, University of Cologne, Cologne, Germany. This study was supported by a grant from Jean Uhrmacher Foundation, Cologne, Germany.

Presented at the meeting of the American Laryngological Association, Palm Desert, California, April 24-25, 1999.

CORRESPONDENCE — Hans Edmund Eckel, MD, Dept of Otorhinolaryngology, University of Cologne, HNO, 50924 Koeln, Germany.

many, from January 1, 1987, to December 31, 1996. The treatment protocol for these patients included transoral laser resection of the primary cancer for lesions classified T1 or T2 that were judged to be endoscopically accessible; conventional vertical or horizontal partial laryngectomy for these lesions if they were not completely accessible endoscopically; total laryngectomy for most lesions classified T3 or T4; and radiotherapy for those patients not suited for, or who rejected, surgery. Four hundred fourteen patients had glottic carcinoma, 239 patients had supraglottic tumors, and 17 had subglottic primaries. Among the 414 patients with glottic carcinoma, 33 were classified Tis, 174 T1, 113 T2, 78 T3, and 16 T4. Out of the 287 patients with glottic lesions classified T1 or T2, 252 were treated with TLS alone. These 252 prospectively treated patients constitute the subject of this investigation.

Of the remaining 35 patients with T1 or T2 glottic lesions, 15 were treated with open partial laryngectomy, 10 had radiotherapy, 7 had total laryngectomy after attempted but unsuccessful partial laryngectomy, 2 had TLS combined with unilateral neck dissection and postoperative radiotherapy for concurrent lymph node metastases, and 1 had no curative treatment.

All patients underwent staging endoscopy of the pharynx, larynx, esophagus, and oral cavity to permit detailed assessment of the tumor and to rule out synchronous coexisting primaries. Patients with clinically negative necks were not offered additional prophylactic treatment for the cervical lymph nodes.

Surgical Technique. The surgical technique for the transoral approach to early-stage larynx carcinoma used throughout this study has previously been described in detail.[8,20,24] Therefore, only a short outline of the surgical procedures used for this study shall be given here. The surgical laser was always coupled to a Zeiss operating microscope and was generally set to an output power of 2 to 6 W in the superpulse mode at a spot size of approximately 0.5 to 0.8 mm^2. All patients were intubated transorally for surgery, except for those who had preexisting tracheostomy. Different laryngoscopes, including bivalved adjustable laryngoscopes as described by Steiner,[10] were used to expose the larynx. For small carcinomas in situ, the mucosa of the vocal cords was resected, leaving the vocal muscle intact. The excision thus corresponded to conventional microlaryngoscopic decortication of the vocal cords. Small, midcordal lesions required a type II resection: one vocal cord was resected completely, leaving the anterior third of the other vocal cord and the arytenoid cartilage intact. The resection equaled a conventional cordectomy in extent of the excision. Tumors with anterior commissure involvement were managed by a type III resection including the anterior commissure down to the thyroid cartilage, to the subglottic region including the cricothyroid membrane, with the upper margin of the cricoid cartilage as a borderline. The extension of the en bloc excision reached up to the arytenoid cartilage and sometimes included it with preservation of the posterior mucosa. The T2 tumors frequently required a type IV resection ("transglottic resection") containing all of the endolaryngeal structures down to the thyroid and cricoid cartilages and to the cricothyroid membrane, as well as to the arytenoid cartilage, which can be included on one side. The cartilaginous framework of the larynx was always left intact. The specimens were resected in one piece and oriented anatomically to facilitate histopathologic examination of the margins.

All patients received 250 mg of methylprednisolone sodium succinate intravenously during the intervention, and 80 mg orally of the same drug during the first days after surgery. Extubation was performed 30 to 60 minutes after the end of the intervention, and the patients were closely observed after extubation.

Statistical Methods. For all 252 patients treated with TLS, the overall survival rate was determined without correction for age or intercurrent death from the date of initiation of therapy (the date of the primary operation) until the date of the last follow-up examination or death. Disease-specific survival and organ preservation rates were calculated likewise. For the cause-specific actuarial survival analysis (tumor-related survival rate), patients who were free of disease and died of unrelated causes were excluded at the time of their death. In addition, these same variables (overall survival, disease-specific survival, and organ preservation rates) were then calculated from the date of the first detection of recurrent disease until the date of the last follow-up examination or death to describe the patients' clinical course after the onset of recurrence. Stratified log-rank tests were used to test for differences between subgroups.

Local recurrence was defined as any manifestation of squamous cell carcinoma in the larynx following initial treatment, regardless of site or time of onset after initial treatment. Regional recurrence was defined as any manifestation of cervical lymph node metastasis from squamous cell carcinoma, if no second primary tumor was identified as the obvious cause of such metastasis. Recurrence at distant sites was defined as any manifestation of squamous cell carcinoma in sites apart from the larynx and cervical lymph nodes if no second primary tumor was identified as

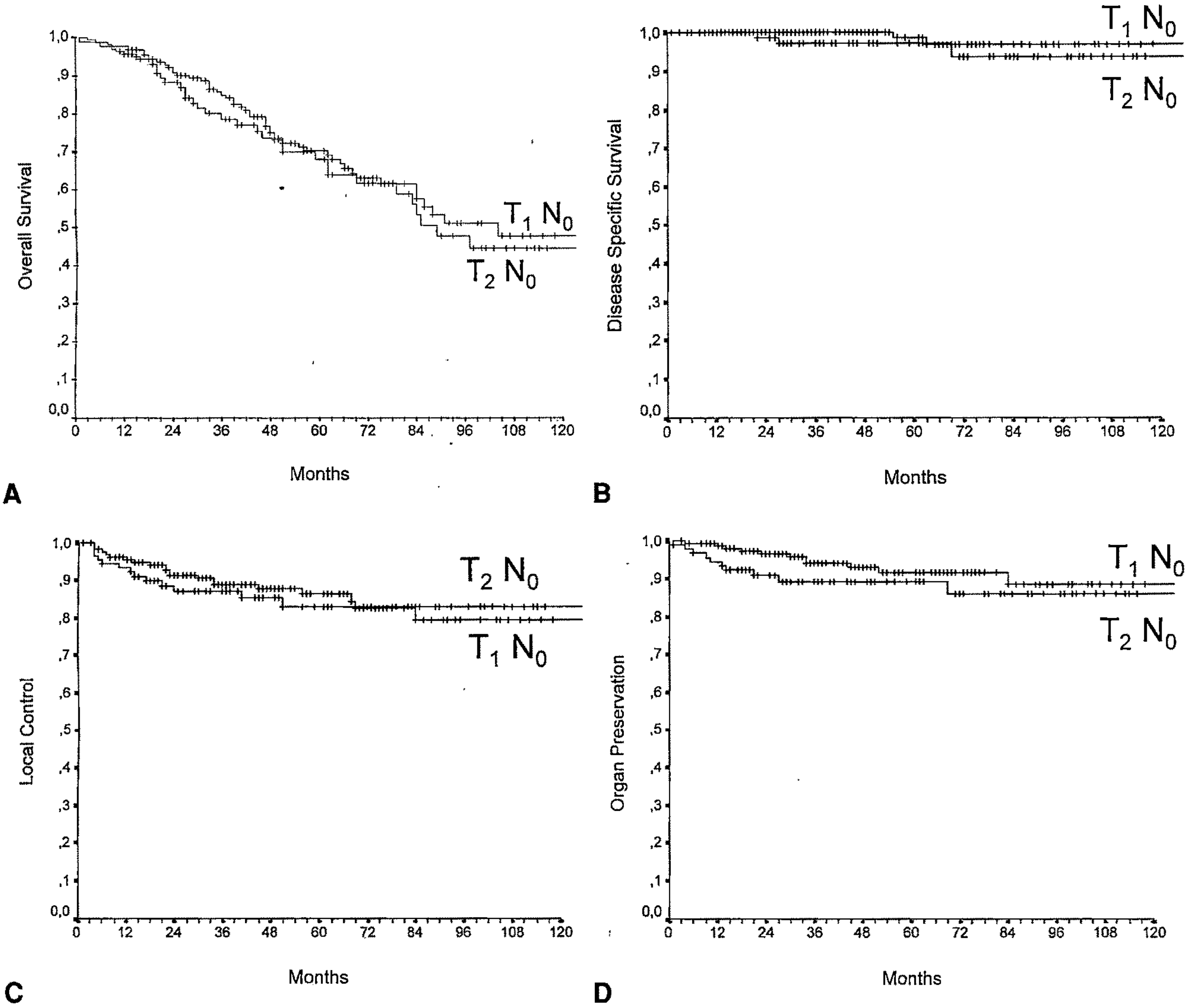


Fig 1. Outcomes for 252 patients treated with transoral laser surgery. A) Overall survival rate. B) Disease-specific survival rate. C) Local control rate. D) Organ preservation rate.

the obvious cause of such tumoral growth.

For all patients included in this study, TNM staging was accomplished according to the fifth edition of the UICC's TNM classification of malignant tumors. The clinical (pretherapeutic) classification, designated cTNM, was used for all cases except for the description of recurrent tumors, for which the so-called re-treatment classification (rTNM) was used.

Follow-up data were available for all patients. Follow-up for this study was continued until December 31, 1997. Patients who survived beyond the end of the data acquisition, or who were lost to follow-up at some point, were excluded at the time of their last follow-up. No patient was excluded from statistical analysis. Follow-up ranged from 12 to 139 months (median, 67 months).

Survival rates were computed by use of the product-limit estimator of Kaplan and Meier. Data were analyzed with the SPSS for Windows (Statistical Package for the Social Sciences) statistical software package version 7.5 (SPSS Inc, Chicago, Ill).

RESULTS

Two hundred fifty-two patients with glottic squamous cell carcinoma were treated with TLS alone from 1987 to 1996. One hundred sixty-one were classified T1N0M0, and 91 T2N0M0. These 252 prospectively treated patients constitute the subject of this investigation. Kaplan Meier curves demonstrating overall survival rates, disease-specific survival rates, local control rates, and organ preservation rates for these patients, stratified according to stage of the disease, are given in Fig 1. The actuarial results for these outcome measures after 5 and 10 years are summarized in the Table. In comparing the results for tumors classified T1N0M0 versus T2N0M0, no significant differences for any of these four outcome

FIVE-YEAR AND TEN-YEAR OUTCOMES FOR 252 PATIENTS TREATED WITH TRANSORAL LASER SURGERY FOR GLOTTIC CARCINOMA

	T1N0M0 (n = 161)		*T2N0M0 (n = 91)*		*p (Log Rank Test)*
	5 y	*10 y*	*5 y*	*10 y*	
Overall survival	70.3	47.7	68.0	44.5	.55
Disease-specific survival	98.6	97.0	97.0	93.6	.22
Local control after initial treatment	86.3	79.2	82.9	82.9	.71
Ultimate local control including salvage treatment	97.0	97.0	97.5	97.5	.71
Organ preservation	91.7	88.5	89.3	86.0	.21

Data are rates in percent.

measures could be detected (log rank test).

Thirty-five patients (13.9%) presented with local recurrences or second laryngeal primaries 4 to 84 months (mean, 23 months; median, 17 months) after initial treatment. Of the 161 patients classified T1N0M0, 21 (13.0%) suffered local recurrences, and of the 91 classified T2N0M0, 14 (15.4%) had tumor recurrences. If tumors recurring more than 60 months after initial treatment are considered second primary tumors rather than recurrences, then only 18 (11.2%) of 161 patients classified T1N0M0 would have had recurrences. However, the difference in local control between patients with stage I versus stage II disease would still not be significant (p = .41).

Of the 35 patients with local recurrences, 16 (45%) were managed with total laryngectomy, 10 (28.6%) with further TLS, 4 (11.4%) with partial laryngectomy, and 2 (5.7%) with radiotherapy, and 3 (8.6%) had no curative treatment. Accordingly, 16 patients (45.7%) with local treatment failure could be treated with further organ-sparing treatment methods.

Kaplan Meier curves demonstrating overall survival, disease-specific survival, and organ preservation rates from the time of local recurrence are given in Fig 2. The actuarial overall survival rate, disease-specific survival rate, and organ preservation rate 5 years after the diagnosis of recurrent disease were

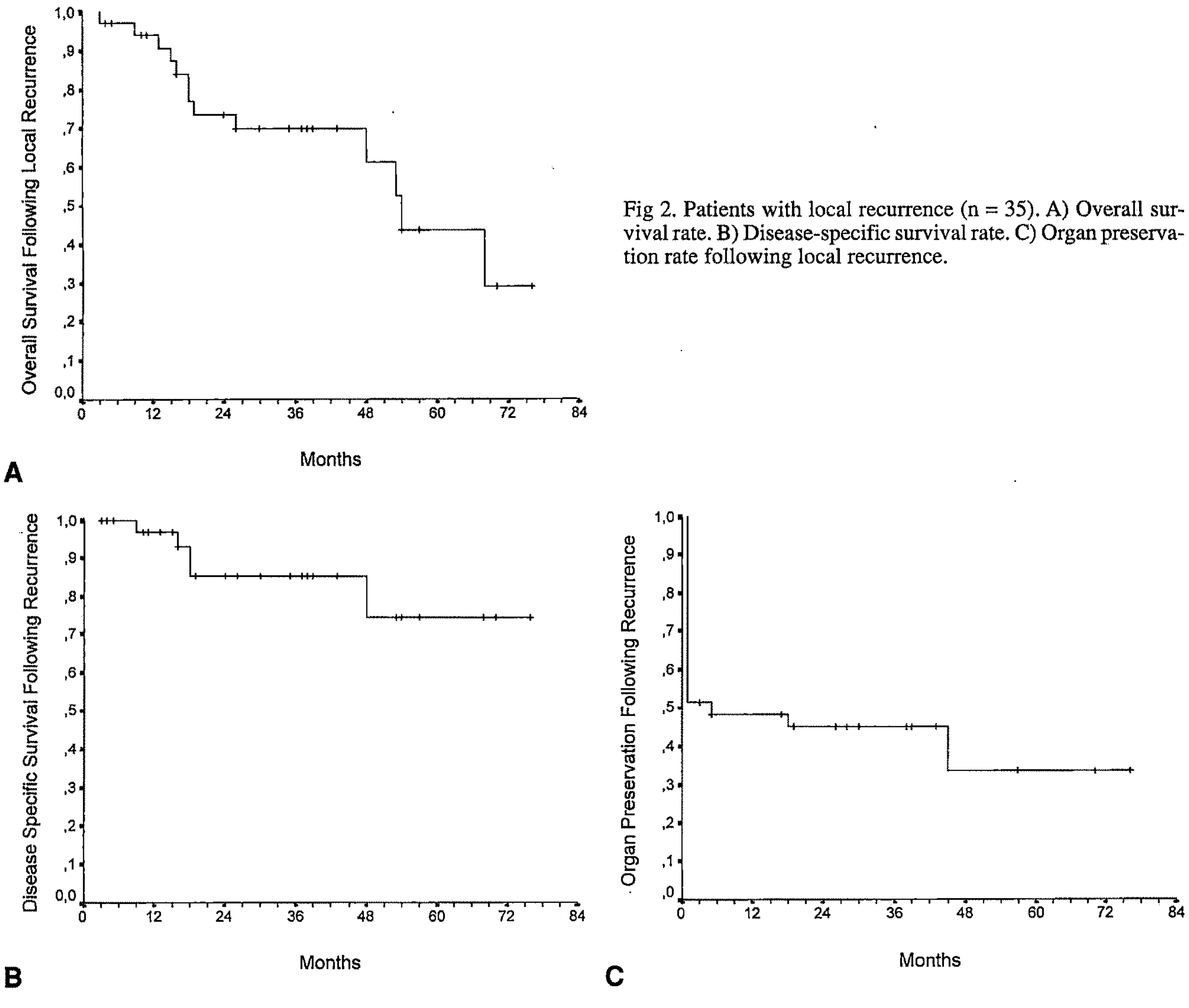


Fig 2. Patients with local recurrence (n = 35). A) Overall survival rate. B) Disease-specific survival rate. C) Organ preservation rate following local recurrence.

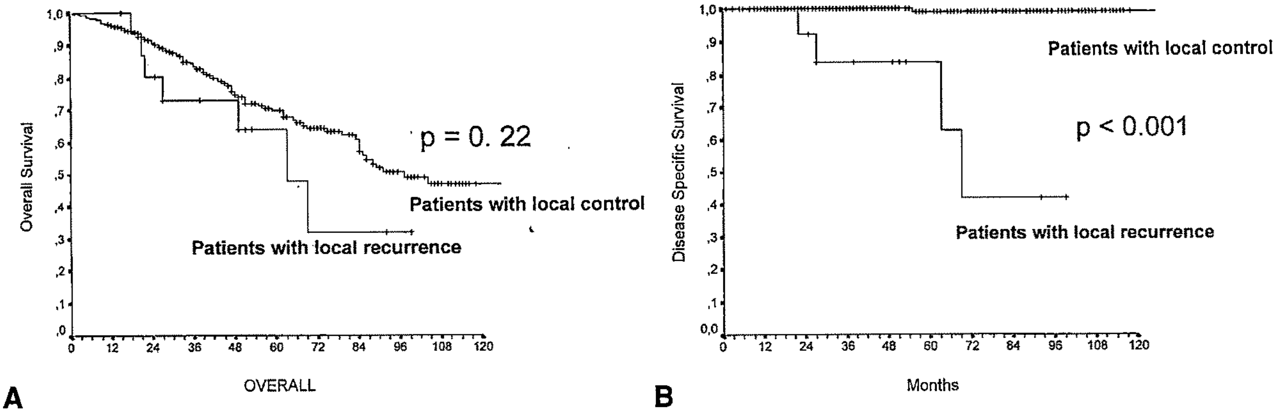


Fig 3. A) Overall survival rate and B) disease-specific survival rate for patients with local control versus patients with local recurrence.

43.6%, 74.6%, and 33.7%.

In addition to the 35 patients with local recurrences described here, 1 patient was found to have lymph node metastases 25 months after the initial TLS, but without local relapse. He was treated with radical neck dissection and postoperative radiotherapy, but died 56 months after initial treatment with uncontrollable regional and distant metastases. No patient with isolated distant metastases without preceding local or regional treatment failure was identified in this study.

Figure 3 plots the overall survival and disease-specific survival rates of those 217 patients with ongoing local control following TLS versus the 35 patients who suffered local recurrences. From these data, the overall survival rate is not significantly worse for patients with local treatment failures. However, the difference in the disease-specific survival rate is highly significant.

The most frequent sites of local recurrences in the larynx were the anterior commissure (n = 13; 37.1%), the vocal cords (n = 11; 31.4%), the subglottis (n = 8; 22.9%), the arytenoid cartilage (n = 5; 14.3%), and other supraglottic structures (n = 4; 11.4%). In some cases, several subsites were involved. Typical clinical examples of such recurrences are given in Figs 4 and 5.

DISCUSSION

For early glottic carcinoma (defined here as T1N0M0 and T2N0M0), excellent local control and survival rates have been reported for radiotherapy and for partial laryngectomy.[2-5,15,25-28] Besides these well-established oncological approaches, TLS has emerged as a major therapeutic alternative during the past 20 years.[7,9,10,13,17-19,24,29] Although endoscopic laser surgery for the treatment of laryngeal neoplasms was first described and used in the United States,[25] it found earlier and wider acceptance in clinical practice in Europe. Initial pioneer work by Steiner[10] and subsequent major studies by Motta et al[13] and Rudert and Werner[9] demonstrated the potential use of TLS for early-stage glottic carcinoma.

Transoral laser surgery provides advantages related to its hemostatic effects and precision of tissue ablation.[11,18,20] It has been reported to cause minimal morbidity, provide good functional results, and provide a cost-effective alternative to open surgical procedures and to radiotherapy.[16,25,30,31] Therefore, it is now a widely used surgical approach to stage I and II glottic carcinoma.[17,21] The data available in the existing body of literature indicate that TLS leads to oncological results that are comparable to those of more conventional treatment methods for these patients.[9,10,13,17,18] However, detailed information on local control rates, patterns of local treatment failure, final organ preservation rates, and outcome following re-treatment for recurrent disease following initial TLS has not been available to date. Such data may be essential for a critical appraisal of the role of TLS for the treatment of early-stage glottic carcinoma, and for a meaningful comparison of TLS to established treatment methods, namely, radiotherapy and open partial laryngectomy.

Local control is clearly the most important single prognostic factor for stage I and II glottic carcinoma since lymph node metastases and distant spread are only rarely observed in the absence of local relapse for these patients. Therefore, curative treatment must obviously strive for high local control rates. On the other hand, quality-of-life issues and the preservation of a functioning larynx are other imperative goals for successful treatment. Otherwise, total laryngectomy, with presumably very high local control rates, would be the ideal treatment option. Therefore, the laryngologist in charge of a patient with early-stage glottic carcinoma has to look for an acceptable com-

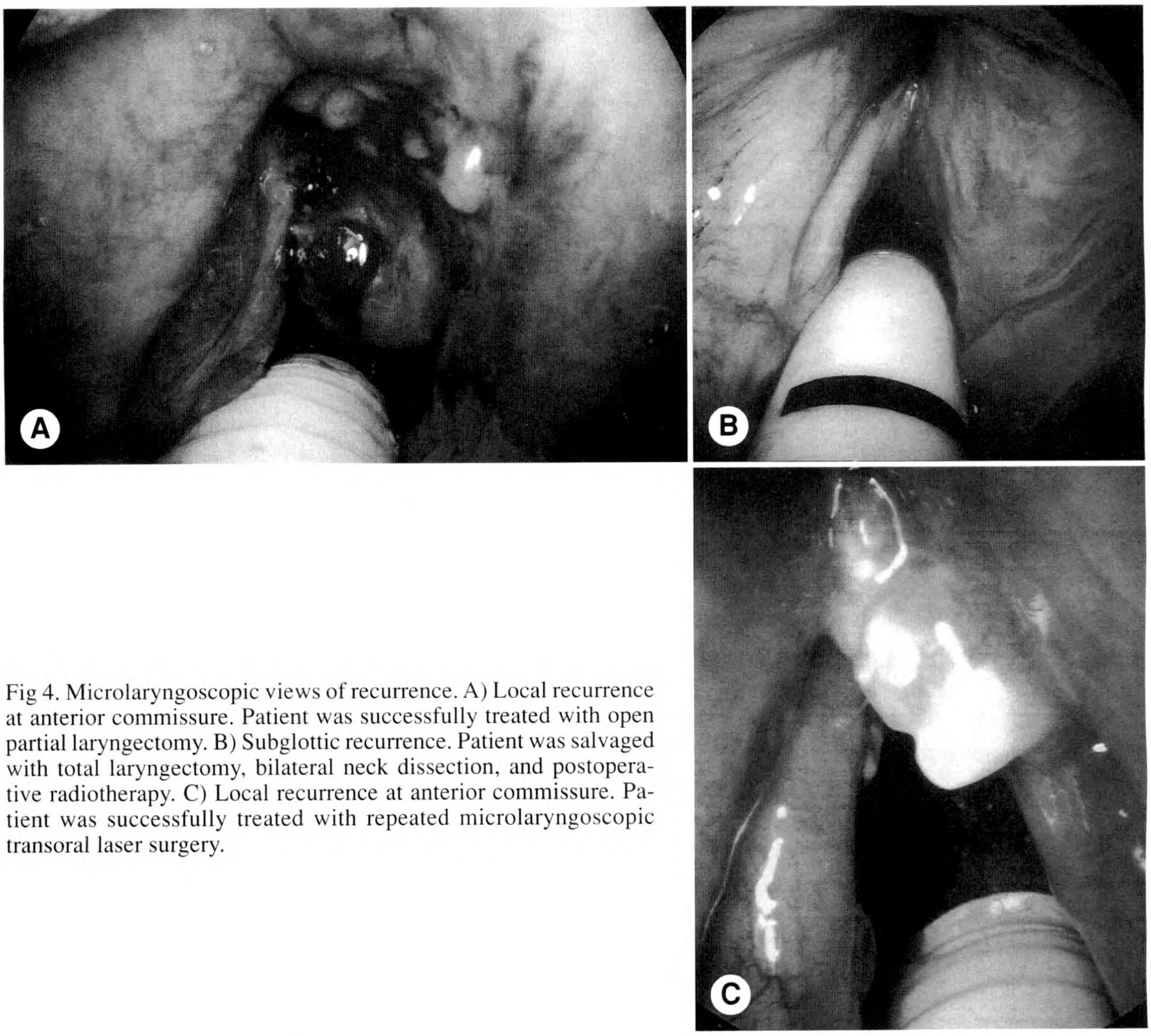


Fig 4. Microlaryngoscopic views of recurrence. A) Local recurrence at anterior commissure. Patient was successfully treated with open partial laryngectomy. B) Subglottic recurrence. Patient was salvaged with total laryngectomy, bilateral neck dissection, and postoperative radiotherapy. C) Local recurrence at anterior commissure. Patient was successfully treated with repeated microlaryngoscopic transoral laser surgery.

promise between local control, morbidity, functional outcome, and cost.

Radiotherapy is now the most frequently used treatment for early glottic carcinoma in the United States, Canada, and Northern Europe.[21,32] Its main advantage is voice preservation, provided the initial treatment is successful. However, the local control rates with radiotherapy as the initial treatment range from 82% to 94% for lesions classified T1 and from 70% to 87% (70% to 80% in most series) for T2 lesions in recent publications[4,26,27,33-35]; these are lower than those reported for partial laryngectomy, for which local control rates of more than 90% have repeatedly been reported for vertical partial procedures and for supracricoid partial laryngectomy.[5,15,25]

For TLS, local control rates ranging from 89% to 94% have so far been reported in the literature for stage I and II glottic carcinoma.[9,10,23] However, not all of these publications had sufficient follow-up of their patients to allow for final conclusions on long-term local control rates.

Optimal results are not always achieved with the above-mentioned approaches. With radiotherapy, local control rates as poor as 18% for T2 lesions have been reported in the literature,[33] and a 40% local failure rate following TLS for T2 glottic cancer has been documented in a large series of patients (n = 158) with sufficient follow-up to determine long-term results.[13] Thomas et al[36] observed a 23% recurrence rate in a series of 106 patients treated with endoscopic surgery for T1 glottic lesions. However, the majority of these cases were not treated with the use of a surgical laser. Sheen et al[37] observed a 14.3% failure rate for early glottic cancer treated with vertical laryngectomy.

In the series of patients presented here, the actuarial local control rate with initial treatment was 86.3% after 5 years and 79.2% after 10 years for T1 lesions and 82.9% after 5 and 10 years for T2 lesions. These findings lie within the range of results reported for initial radiotherapy in the recent literature. They are comparable to those following radiotherapy for lesions classified T1, and slightly better

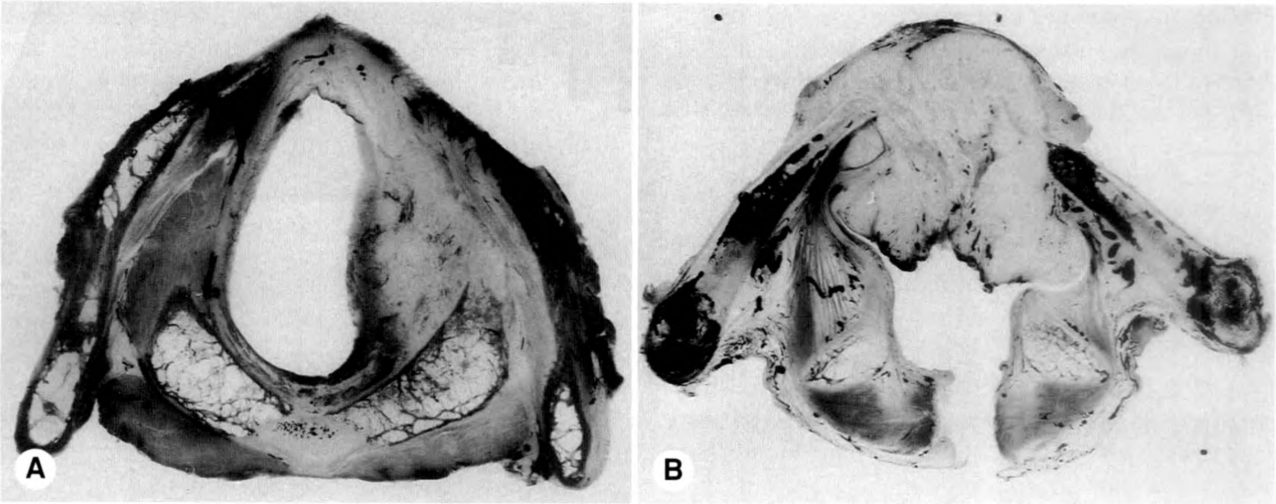


Fig 5. Whole organ sections of larynges with recurrent carcinoma. A) Recurrence in subglottic region following initial laser surgery for T1N0M0 vocal cord carcinoma. B) Recurrence in anterior commissure following initial laser surgery for T2N0M0 vocal cord carcinoma.

than those following radiotherapy for lesions classified T2.[2,4,26,27,33-35] On the other hand, local control rates for conventional partial laryngectomy have been reported to be superior to those documented in this study with laser surgery.[5,15,25] Because no prospective randomized trials on local control rates of different treatment methods for early-stage glottic carcinoma have been conducted so far, comparative estimations of the true ability to achieve long-term local control between different treatment methods still remain to be assessed.

However, if local recurrence occurs, then more retreatment options are available after initial laser surgery as compared to initial radiotherapy or open surgery, since wound complications as a result of previous irradiation are not encountered and the laryngeal framework is still intact, preventing early extralaryngeal spread of local failures. Therefore, such recurrences allow for further organ-sparing procedures if they are discovered in a timely way. In such situations, all treatment options are still available: further endolaryngeal surgery, conventional partial laryngectomy, radiotherapy, or total laryngectomy. However, meticulous follow-up of these patients is required if local recurrences are to be discovered early enough for further voice-sparing therapy. Organ preservation therapy for local recurrences was possible in 16 patients (45.7% of all patients with local treatment failure) in this series of patients. It resulted in a 33.7% rate of preserving the larynx 5 years after treatment for recurrent disease (Fig 2C). Innovations in the field of laser technology and surgical technique may contribute to improved oncological results in the future.[38-42]

The anterior commissure was most frequently affected when local recurrences occurred in the larynx. It was involved in 13 (37.1% of all patients) of the 35 cases of local recurrence described here. That the anterior commissure is the subsite most frequently at risk of giving rise to local treatment failure holds true for initial radiotherapy, most conventional partial laryngectomies, and for TLS, as well.[22,43,44] Therefore, it is not surprising that local recurrences were more frequently noted here than at any other subsite of the larynx.

For this reason, endoscopic laser resection usually has been discouraged for anterior commissure carcinoma because of inadequate exposure and close proximity to underlying cartilage, and the results presented here confirm that these restraints are truly justified. As a consequence of these clinical observations, the surgical approach to anterior commissure carcinoma has recently changed at the author's institution. Supracricoid laryngectomy with cricohyoidoepiglottopexy is now used for these lesions, since consistent data in the literature demonstrate that this technique can achieve local control rates that are clearly superior to those reported here.[15]

Another new technique combining endoscopic CO_2 laser incision and an external approach, creating a window in the thyroid cartilage, has recently been published by Shapshay et al[42] and Rebeiz et al.[45] It is believed that this new combined technique may overcome the limited access obtained with the endoscopic technique. It may therefore become a successful alternative to purely endoscopic surgery for anterior commissure lesions.[42,45] However, the author has no personal experience with this innovative technique to date.

The overall survival rates in this study clearly illustrate that patients with glottic carcinoma are usually more than 60 years old at the time of initial diagnosis, and that life expectancy therefore is limited. In contrast, cause-specific survival rates for all T categories under investigation in this study show very high disease-specific survival rates, leaving little room for further improvement in final outcome: T1 and T2 glottic carcinoma seem to bear a largely identical prognosis if they are treated with TLS.

CONCLUSION

Although favorable local control rates and re-treatment results in cases of local failure for early larynx carcinoma treated with laser surgery are documented in this study, no definite recommendation on the best single treatment method can be given in this report. As evidenced in the literature, conventional partial laryngectomy leads to the highest local control rates reported so far, radiotherapy is believed to preserve voice best, and laser surgery is associated with time- and cost-effectiveness, a low level of morbidity, fair local control rates, and excellent re-treatment options for local failure. In the near future, innovations in the field of laser technology and surgical technique may contribute to even more reliable tumor ablation and improved local control rates.

However, further prospective studies will be needed to determine the functional outcome for these different treatment options, especially in terms of voice, airway, and deglutition. In light of the recent literature on treatment for larynx carcinoma, the future may bring new roles for surgery and radiotherapy. Surgery, including TLS, may become a more widespread approach to early-stage disease, while organ preservation programs based on sequential or concomitant chemotherapy and radiotherapy may replace surgery as the most important treatment method for advanced but resectable stages. Therefore, all specialists dealing with larynx carcinoma should be able to offer these different treatments to their patients, and to respond specifically to their patients' individual needs and preferences.

ACKNOWLEDGMENTS — The author thanks H. Stützer, MD, Department of Biostatistics, University of Cologne, for his advice on the statistical methods used in this study. Christian Sittel, MD, and Sabine Ricke prepared the plastinated whole organ section displayed in Fig 5A.

REFERENCES

1. Johnson JT, Myers EN, Hao S-P, Wagner RL. Outcome of open surgical therapy for glottic carcinoma. Ann Otol Rhinol Laryngol 1993;102:752-5.

2. Kaplan MJ, Johns ME, Clark DA, Cantrell RW. Glottic carcinoma. The roles of surgery and irradiation. Cancer 1984; 53:2641-8.

3. Lusinchi A, Dube P, Wibault P, Kunkler I, Luboinski B, Eschwege F. Radiation therapy in the treatment of early glottic carcinoma: the experience of Villejuif. Radiother Oncol 1989; 15:313-9.

4. Mendenhall WM, Parsons JT, Stringer SP, Cassisi NJ, Million RR. T1-T2 vocal cord carcinoma: a basis for comparing the results of radiotherapy and surgery. Head Neck Surg 1988;10:373-7.

5. Olsen KD, Thomas JV, DeSanto LW, Suman VJ. Indications and results of cordectomy for early glottic carcinoma. Otolaryngol Head Neck Surg 1993;108:277-82.

6. Ambrosch P, Kron M, Steiner W. Carbon dioxide laser microsurgery for early supraglottic carcinoma. Ann Otol Rhinol Laryngol 1998;107:680-8.

7. Czigner J, Savay L. Primary CO_2 laser cordectomy in vocal cord carcinoma. Laryngorhinootologie 1994;73:432-6.

8. Damm M, Eckel HE, Schneider D, Arnold G. CO_2 laser surgery for verrucous carcinoma of the larynx. Lasers Surg Med 1997;21:117-23.

9. Rudert HH, Werner JA. Endoscopic resections of glottic and supraglottic carcinomas with the CO_2 laser. Eur Arch Otorhinolaryngol 1995;252:146-8.

10. Steiner W. Results of curative laser microsurgery of laryngeal carcinomas. Am J Otolaryngol 1993;14:116-21.

11. Zeitels SM, Vaughan CW, Domanowski GF. Endoscopic management of early supraglottic cancer. Ann Otol Rhinol Laryngol 1990;99:951-6.

12. DeSanto LW, Olsen KD, Perry WC, Rohe DE, Keith RL. Quality of life after surgical treatment of cancer of the larynx. Ann Otol Rhinol Laryngol 1995;104:763-9.

13. Motta G, Esposito E, Cassiano B, Motta S. T1-T2-T3 glottic tumors: fifteen years experience with CO_2 laser. Acta Otolaryngol Suppl (Stockh) 1997(suppl 527):155-9.

14. Eckel HE. Endoscopic laser resection of supraglottic carcinoma. Otolaryngol Head Neck Surg 1997;117:681-7.

15. Chevalier D, Laccourreye O, Brasnu D, Laccourreye H, Piquet JJ. Cricohyoidoepiglottopexy for glottic carcinoma with fixation or impaired motion of the true vocal cord: 5-year oncologic results with 112 patients. Ann Otol Rhinol Laryngol 1997; 106:364-9.

16. Myers EN, Wagner RL, Johnson JT. Microlaryngoscopic surgery for T1 glottic lesions: a cost-effective option. Ann Otol Rhinol Laryngol 1994;103:28-30.

17. Eckel HE, Schneider C, Jungehülsing M, Damm M, Schröder U, Vössing M. Potential role of transoral laser surgery for larynx carcinoma. Lasers Surg Med 1998;23:79-86.

18. Ossoff RH, Coleman JA, Courey MS, Duncavage JA, Werkhaven JA, Reinisch L. Clinical applications of lasers in otolaryngology–head and neck surgery. Lasers Surg Med 1994; 15:217-48.

19. Remacle M, Lawson G, Jamart J, Minet M, Watelet JB, Delos M. CO_2 laser in the diagnosis and treatment of early cancer of the vocal fold. Eur Arch Otorhinolaryngol 1997;254:169-76.

20. Rudert H. Technique and results of transoral laser surgery for small vocal cord carcinomas. Adv Otorhinolaryngol 1995;49:222-6.

21. Shah JP, Karnell LH, Hoffman HT, et al. Patterns of care

for cancer of the larynx in the United States. Arch Otolaryngol Head Neck Surg 1997;123:475-83.

22. Eckel HE. Topographical and clinico-oncologic analysis of locoregional recurrence after transoral laser surgery for laryngeal cancer. Laryngorhinootologie 1993;72:406-11.

23. Thumfart WF, Eckel HE, Sprinzl GM. Analysis of recurrences after transoral laser resection of larynx carcinomas. Adv Otorhinolaryngol 1995;49:245-9.

24. Eckel HE, Thumfart WF. Laser surgery for the treatment of larynx carcinomas: indications, techniques, and preliminary results. Ann Otol Rhinol Laryngol 1992;101:113-8.

25. Thomas JV, Olsen KD, Neel HB III, DeSanto LW, Suman VJ. Early glottic carcinoma treated with open laryngeal procedures. Arch Otolaryngol Head Neck Surg 1994;120:264-8.

26. Fein DA, Mendenhall WM, Parsons JT, Million RR. T1-T2 squamous cell carcinoma of the glottic larynx treated with radiotherapy: a multivariate analysis of variables potentially influencing local control. Int J Radiat Oncol Biol Phys 1993;25:605-11.

27. Medini E, Medini A, Gapany M, Levitt SH. Radiation therapy in early carcinoma of the glottic larynx T1N0M0. Int J Radiat Oncol Biol Phys 1996;36:1211-3.

28. Medini E, Medini I, Lee CK, Gapany M, Levitt SH. Curative radiotherapy for stage II-III squamous cell carcinoma of the glottic larynx. Am J Clin Oncol 1998;21:302-5.

29. Strong MS, Jako GJ. Laser surgery in the larynx. Early clinical experience with continuous CO2 laser. Ann Otol Rhinol Laryngol 1972;81:791-8.

30. Sittel C, Eckel HE, Eschenburg C, Vossing M, Pototschnig C, Zorowka P. Voice quality following endolaryngeal laser surgery. Laryngorhinootologie 1998;77:219-25.

31. Sittel C, Eckel HE, Eschenburg C. Phonatory results following laser surgery for glottic carcinoma. Otolaryngol Head Neck Surg 1998;119:418-24.

32. O'Sullivan B, Mackillop W, Gilbert R, et al. Controversies in the management of laryngeal cancer: results of an international survey of patterns of care. Radiother Oncol 1994;31:23-32.

33. Kanonier G, Rainer T, Fritsch E, Thumfart WF. Radiotherapy in early glottic carcinoma. Ann Otol Rhinol Laryngol 1996;105:759-63.

34. Reddy SP, Mohideen N, Marra S, Marks JE. Effect of tumor bulk on local control and survival of patients with T1 glottic cancer. Radiother Oncol 1998;47:161-6.

35. Mendenhall WM, Parsons JT, Stringer SP, Cassisi NJ. Management of Tis, T1, and T2 squamous cell carcinoma of the glottic larynx. Am J Otolaryngol 1994;15:250-7.

36. Thomas JV, Olsen KD, Neel HB III, DeSanto LW, Suman VJ. Recurrences after endoscopic management of early (T1) glottic carcinoma. Laryngoscope 1994;104:1099-104.

37. Sheen T-S, Ko J-Y, Chang Y-L. Partial vertical laryngectomy in the treatment of early glottic cancer. Ann Otol Rhinol Laryngol 1998;107:593-7.

38. Pearson BW, DeSanto LW, Olsen KD, Salassa JR. Results of near-total laryngectomy. Ann Otol Rhinol Laryngol 1998;107:820-5.

39. Howard J, Arango P, Ossoff J, Ossoff RH, Reinisch L. Healing of laser incisions in rat dermis: comparisons of the carbon dioxide laser under manual and computer control and the scalpel. Lasers Surg Med 1997;20:90-6.

40. Reinisch L, Mendenhall MH, Ossoff RH. Precise laser incisions, corrected for patient respiration with an intelligent aiming system. Lasers Surg Med 1997;20:210-5.

41. Zeitels SM, Vaughan CW. "External counterpressure" and "internal distention" for optimal laryngoscopic exposure of the anterior glottal commissure. Ann Otol Rhinol Laryngol 1994;103:669-75.

42. Shapshay SM, Wang Z, Rebeiz EE, Perrault DF Jr, Pankratov MM. A combined endoscopic CO2 laser and external approach for treatment of glottic cancer involving the anterior commissure: an animal study. Laryngoscope 1996;106:273-9.

43. Kirchner JA. What have whole organ sections contributed to the treatment of laryngeal cancer? Ann Otol Rhinol Laryngol 1989;98:661-7.

44. Rucci L, Gammarota L, Gallo O. Carcinoma of the anterior commissure of the larynx: II. Proposal of a new staging system. Ann Otol Rhinol Laryngol 1996;105:391-6.

45. Rebeiz EE, Wang Z, Annino DJ, McGilligan JA, Shapshay SM. Preliminary clinical results of window partial laryngectomy: a combined endoscopic and open technique. Ann Otol Rhinol Laryngol 2000;109:123-7.

Ann Otol Rhinol Laryngol 110:2001

ACQUIRED TOTAL (GRADE 4) SUBGLOTTIC STENOSIS IN CHILDREN

L. MARK GUSTAFSON, MD
BENJAMIN E. J. HARTLEY, MD ROBIN T. COTTON, MD
CINCINNATI, OHIO

Pediatric acquired total subglottic stenosis (SGS) is a challenging problem. The management of these patients has evolved at our institution over the past 25 years. We conducted a retrospective study to evaluate the surgical management and outcomes of children with grade 4 SGS. Fifty-six patients have presented with acquired grade 4 SGS since 1981. The causes included previous surgery (34), prolonged intubation (15), burns (1), and unknown causes (6). Of the 56 patients, 44 (79%) were decannulated; 120 total procedures were performed, and 39 patients (70%) required more than 1 procedure for decannulation. The decannulation rate has risen from 67% in the 1980s to 86% in the 1990s. Patients who underwent cricotracheal resection (CTR) had a higher decannulation rate than patients who underwent laryngotracheal reconstruction (LTR) with anterior and posterior costal cartilage grafting (CCG) (92% versus 81%), and were less likely to need additional open procedures to achieve decannulation (18% versus 46%). The decannulation rate for children with grade 4 SGS has improved because of advances in surgical technique. Currently, the principal operations used at our institution are 1) CTR and 2) LTR with anterior and posterior CCG. There was a trend toward a higher decannulation rate in patients who underwent CTR, and they were less likely to require further reconstructive surgery before decannulation.

KEY WORDS — cricotracheal resection, laryngotracheal reconstruction, subglottic stenosis.

INTRODUCTION

The management of severe subglottic stenosis (SGS) in children is difficult, and a wide variety of surgical techniques have been developed to provide these patients with a patent and functional airway. Although the majority of mild or moderate cases of SGS can be successfully corrected with single-stage laryngotracheal reconstruction (LTR),[1-3] severe SGS remains a difficult challenge. These patients often require multistaged procedures, and successful decannulation may sometimes be achieved only after a series of operations. Recent reports of cricotracheal resection (CTR) for severe SGS in children have cited promising results[4-6]; however, no single approach has been reported to be universally successful.

Even more challenging is the management of patients who have no laryngeal lumen at all. Children with acquired total SGS (Cotton grade 4)[7] represent a unique population. With no anatomic lumen to expand, surgery must focus on first creating an opening and then maintaining a widened lumen. Often, the stenoses represent thick cicatricial scar bands formed by traumatic injury to the subglottis. Originally, many of these children were condemned to a permanent tracheostomy with no voice and a significant risk of airway compromise if the tracheostomy tube became obstructed with secretions. Over the past several decades, the management of these patients has evolved. With the introduction of new surgical techniques, fewer operations are required, and the success of these procedures has increased. This study reviews our experience with acquired complete SGS in children and outlines our current approach to the surgical management of these patients.

METHODS

Since 1981, the pediatric otolaryngology department at Children's Hospital, Cincinnati, has maintained an ongoing database of all operative airway procedures. A retrospective review was undertaken to identify all children who presented with acquired complete (Cotton grade 4) SGS. Cotton grade 4 stenosis is defined as the lack of any identifiable lumen or fistula in the subglottis.[7] Patients with laryngeal atresia, congenital subglottic stenosis, or tracheal atresia were specifically excluded from this study. Only those children with at least 1 year of follow-up were included in our analysis.

Patient demographics were collected from the database and patient charts. The cause of the SGS was identified as prolonged intubation, previous surgery, burns, or unknown causes. The number and type of prior airway operations were noted. The initial procedure performed by our department to correct the stenosis was recorded, along with any additional procedures performed after that initial surgery. The decannulation rates, time to decannulation, and number of operations required were also recorded. Statis-

---

From the Department of Otolaryngology–Head and Neck Surgery, Children's Hospital Medical Center, Cincinnati, Ohio.

Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

CORRESPONDENCE — L. Mark Gustafson, MD, Dept of Otolaryngology–Head and Neck Surgery, University of Cincinnati Medical Center, 231 Bethesda Ave, PO Box 670528, Cincinnati, OH 45267-0528.

INITIAL OPERATIVE PROCEDURES

Initial Procedure	*1980s*	*1990s*
LTR with anterior and posterior CCG	5	10
Cricotracheal resection	0	12
LTR with anterior and posterior split	6	3
LTR with posterior CCG	4	6
Four-quadrant split	2	1
LTR with anterior CCG	3	0
LTR with anterior CCG and posterior split	1	3

Types of operations performed are listed with number of times in each decade they were used as initial procedure to correct grade 4 subglottic stenosis. LTR — laryngotracheal reconstruction, CCG — costal cartilage graft.

tical analysis was performed with χ^2 tests for categorical data.

RESULTS

Fifty-six patients have presented with acquired grade 4 SGS since 1981. Twenty-one were initially treated in the 1980s, and 35 have been seen since 1990. There were 24 girls and 32 boys. The mean age was 4.8 years (range, 2 months to 16.8 years). The causes of the stenoses included prolonged intubation (15), previous surgery (34), unknown causes (6), and burns (1).

The initial operations performed to correct the grade 4 SGS are listed in the Table. In the 1980s, LTR with anterior and posterior division of the trachea (n = 5), LTR with anterior and posterior costal cartilage grafting (CCG) (n = 5), and LTR with posterior CCG (n = 4) were the most common initial surgical procedures. In the 1990s, CTR was introduced, and it has become the predominant method of correcting complete SGS (n = 12). The LTR with anterior and posterior CCG (n = 10) remained frequent, followed by LTR with posterior CCG (n = 6).

Of the 56 patients, 44 (79%) have been decannulated. A total of 120 procedures were performed. Eighteen patients were decannulated after 1 procedure, 12 after 2 procedures, 6 after 3 procedures, 2 after 4 procedures, 3 after 5 procedures, and 1 each after 6, 7, and 9 procedures. The mean time to decannulation was 28 months (range, 0.1 to 120 months). The decannulation rate in the 1980s was 67%, and that in the 1990s was 86% ($p = .02$).

Thirteen of the 16 patients (81%) who underwent LTR with anterior and posterior CCG have been decannulated. Six of those patients (46%) required more than 1 open airway procedure in order to be decannulated. Eleven of the 12 patients (92%) who underwent CTR have been decannulated ($p = .61$). Only 2 of the 11 patients (18%) required an additional operation before being decannulated ($p = .15$).

DISCUSSION

The treatment of complete SGS in children remains a difficult challenge. Although LTR with cartilage augmentation of the tracheal lumen has become a well-established means of treating moderate to severe subglottic stenosis,[3-12] the correction of grade 4 stenosis is more problematic. Many authors have published their experience with the surgical management of pediatric laryngotracheal stenosis.[3,8,9,11] However, few studies have focused on the management of complete SGS, and results from large series often combine patients with various grades of stenosis, making an analysis of the patients with grade 4 stenosis difficult. In this study, we examined the largest reported series of children with acquired grade 4 SGS to review our approach to this challenging patient population and to determine whether the introduction of new surgical techniques has resulted in improved outcomes.

The majority of our patients (60%) developed complete stenosis after some type of surgical intervention in their airway. Often, this involved either dilation of the tracheal lumen or use of a laser to ablate a stenosis. In each of these cases, an incomplete stenosis progressed to a complete stenosis after the procedure. Surgeons need to be cognizant of this risk when operating in the subglottis, especially if that intervention may traumatize the area in a concentric fashion. Six of the patients in whom complete SGS developed secondary to surgery underwent failed attempts to correct the complete stenosis at other institutions. Complete SGS redeveloped in all 6 children. Prolonged intubation was also a major cause (27%). Any form of trauma to the tracheal mucosa can lead to erosion of that protective lining and result in granulation tissue, scarring, and stenosis. A preexisting narrowing of the subglottis may increase the chance of progression to complete stenosis.

The overall decannulation rate in our series was 79%. This compares favorably with other reported rates for grade 4 patients, which have ranged from 25% to 90%.[5,8-10,12] Of significance, however, is that only 18 of the 44 decannulated patients (41%) were able to be decannulated after 1 procedure. Most of the remaining children required 1 additional operation, although several needed multiple operations. In addition, those patients who have not been decannulated have often undergone numerous operations in an attempt to maintain an adequate airway.

Overall, 120 operations have been performed on the 56 patients in our series. In an extensive review of his experience treating pediatric SGS, Cotton[9] reported that 83% of patients with grade 2 stenosis and 81% with grade 3 stenosis were decannulated after 1 procedure. The greater number of procedures required in patients with grade 4 stenosis reflects the fact that often an initial procedure is needed to create a lumen that frequently then requires further augmentation through additional surgery.

The rate of successful outcome in our series has risen in the last decade. The decannulation rate was 67% in the 1980s, but it increased to 86% in the 1990s. This improvement reflects not only an increased experience in treating these patients, but also an evolution in the surgical techniques employed. In addition, the role that gastroesophageal reflux plays in the creation and management of SGS has been better elucidated over the past decade.[13,14] All of our patients now undergo pH probe testing before their surgery and are treated with appropriate medical and surgical therapy to control their reflux both before and after their procedure. The effect of this treatment plan on the success of our surgeries is being investigated currently.

Single-stage procedures with endotracheal tube stenting have become a standard method of correcting less severe pediatric SGS.[1-3] We have rarely used this method for our patients with grade 4 stenosis, because of the severity of their disease process and the presence of other pulmonary or cardiac conditions. Six of our patients had their initial operation performed in a single stage with short-term endotracheal tube stenting; 1 of them underwent a CTR. Only 2 of the 6 were able to be extubated successfully without further surgical intervention or a tracheostomy.

During the 1980s, the concept of augmentation laryngotracheoplasty for pediatric patients gained wide acceptance. Successful experience with the use of cartilage augmentation to widen the tracheal lumen was reported in several large series, and numerous variations on this basic approach were developed.[8,9,11,12] This approach is reflected in our series by the types of initial operations performed during the 1980s (see Table). The placement of anterior and/or posterior grafts predominated, along with other tracheal expansion techniques such as LTR with anterior and posterior division of the cricoid, or a 4-quadrant division of the cricoid.

In 1993, Monnier et al[4] reported their results with CTR in children. Initial concerns that partial cricoid resection with tracheal anastomosis would risk dehiscence and potentially interfere with laryngeal growth have been answered,[4-6] and CTR is rapidly becoming a standard method of treating severe pediatric SGS. This represents a different approach to SGS by focusing on resection, as opposed to expansion, of the stenotic segment.

The majority of our patients with grade 4 SGS now undergo either CTR or LTR with anterior and posterior CCG. Sixteen patients have undergone LTR with anterior and posterior CCG, and 81% have been decannulated. The use of CTR has resulted in a higher decannulation rate (92%), and fewer patients (18%) require a second operation to achieve that result. The 1 patient who underwent CTR and has not been decannulated is able to plug his tracheostomy tube day and night without difficulty and likely will be decannulated soon.

Our series, although the largest one of children with grade 4 stenosis reported to date, is small, because of the rarity of this condition. The small numbers limit the significance of our statistical analysis. The current data, however, support the conclusion that when used appropriately, CTR and LTR with anterior and posterior CCG for grade 4 SGS can result in a high rate of decannulation. Cricotracheal resection, in particular, may minimize the number of operations required for decannulation.

Choosing the appropriate operation for the patient is critical to a successful outcome. Laryngotracheal resection with anterior and posterior CCG has been used for many years to treat severe SGS. Its advantages include the fact that most pediatric otolaryngologists who treat SGS are comfortable with the procedure. In addition, the grafts can be placed up to the vocal cords or within a stenotic interarytenoid area. The ability to adjust the placement of the grafts increases its usefulness for treating multilevel stenosis. The use of 2 interpositional grafts, however, can contribute to instability of the surgical site. Graft prolapse and restenosis may increase the likelihood that long-term stenting will be required to allow a stable expanded subglottis to form.

Cricotracheal resection avoids the use of interpositional grafts by resecting the stenotic segment. That the repair is a single primary anastomosis increases our ability to perform CTR as a single-stage procedure. Monnier et al[5] were able to perform a single-stage CTR in 21 of 38 patients. Cricotracheal resection also preserves the normal laryngeal framework by avoiding a posterior cricoid split or graft. In addition, CTR reduces the donor site morbidity associated with the harvesting of rib cartilage.

There are several disadvantages to CTR. It is a relatively new procedure for pediatric SGS, and therefore, most otolaryngologists are not as comfortable with it as they are with more conventional LTR. In addition, CTR may not be as useful for stenoses near the vocal cords. Walner et al[15] reported that the stenosis should ideally be at least 3 mm from the inferior surface of the cords. We have performed CTR on patients whose stenosis approached the undersurface of the vocal cords and have combined CTR with a posterior CCG in a few patients. The initial results have been encouraging, but further experience is necessary. There is also a small but real risk of anastomosis dehiscence (2% to 4% in reported series). Suprahyoid release of the trachea and chin-to-chest sutures may be used to eliminate tension on the anastomotic site.

At this time, the majority of our patients with grade 4 SGS undergo either a CTR or an LTR with anterior and posterior CCG. In children with other significant medical problems who will require a double-stage procedure, LTR with anterior and posterior CCG with a tracheostomy and suprastomal stent is often performed. T-tubes are most often used for long-term stenting after CTR, and in children with multiple other problems, tolerance and care of a T-tube can be difficult. Cricotracheal resection has primarily been employed as a salvage procedure for those children in whom multiple prior open procedures have failed and as a primary procedure for children with stenoses adequately below the vocal cords. For stenoses near the cords, LTR with anterior and posterior CCG is the most common procedure, although we are gaining more experience with CTR in these children as well.

Relative contraindications for LTR with anterior and posterior CCG are a history of multiple prior LTRs with CCG that have failed and the lack of an adequate amount of costal cartilage left to harvest. Ultimately, however, there are no rigid algorithms that should be followed when deciding what surgery to perform. The relative merits of each procedure, the anatomy of the lesion, and the medical, social, and emotional status of the patient must all be considered.

CONCLUSION

We report a series of 56 children treated with complete acquired SGS. The majority developed their stenosis after prior surgical procedures. Seventy-nine percent have been decannulated. However, the majority of patients required more than 1 operation in order to be decannulated. The decannulation rates have risen significantly in the past decade, and this improvement corresponds to the use of CTR to treat these patients. Of the children who underwent CTR, 92% have been decannulated, and 82% of them required only 1 procedure.

The treatment of children with complete acquired SGS remains challenging. The development of new surgical techniques like CTR, however, has improved our ability to successfully decannulate these children in a timely manner.

REFERENCES

1. Lusk RP, Gray S, Muntz HR. Single-stage laryngotracheal reconstruction. Arch Otolaryngol Head Neck Surg 1991; 117:171-3.

2. Seid AB, Pransky SM, Kearns DB. One-stage laryngotracheoplasty. Arch Otolaryngol Head Neck Surg 1991;117:408-10.

3. Cotton RT, Myer CM III, O'Connor DM, Smith ME. Pediatric laryngotracheal reconstruction with cartilage grafts and endotracheal tube stenting: the single-stage approach. Laryngoscope 1995;105:818-21.

4. Monnier P, Savary M, Chapuis G. Partial cricoid resection with primary anastomosis for subglottic stenosis in infants and children. Laryngoscope 1993;103:1273-83.

5. Monnier P, Lang F, Savary M. Partial cricotracheal resection for severe pediatric subglottic stenosis: update of the Lausanne experience. Ann Otol Rhinol Laryngol 1998;107:961-8.

6. Stern Y, Gerber ME, Walner DL, Cotton RT. Partial cricotracheal resection with primary anastomosis in the pediatric age group. Ann Otol Rhinol Laryngol 1997;106:891-6.

7. Ochi JW, Evans JNG, Bailey CM. Pediatric airway reconstruction at Great Ormond Street: a ten-year review. I. Laryngotracheoplasty and laryngotracheal reconstruction. Ann Otol Rhinol Laryngol 1992;101:465-8.

8. Cotton RT, Gray SD, Miller RP. Update of the Cincinnati experience in pediatric laryngotracheal reconstruction. Laryngoscope 1989;99:1111-6.

9. Cotton RT. The problem of pediatric laryngotracheal stenosis: a clinical and experimental study on the efficacy of autogenous cartilaginous grafts placed between the vertically divided halves of the posterior lamina of the cricoid cartilage. Laryngoscope 1991;101(suppl 56).

10. Narcy P, Contencin P, Fligny I, Francois M. Surgical treatment of laryngotracheal stenosis in the pediatric patient. Arch Otolaryngol Head Neck Surg 1990;116:1047-50.

11. Zalzal GH. Treatment of laryngotracheal stenosis with anterior and posterior cartilage grafts. Arch Otolaryngol Head Neck Surg 1993;119:82-6.

12. Cotton RT, Mortelliti AJ, Myer CM III. Four-quadrant cricoid cartilage division in laryngotracheal reconstruction. Arch Otolaryngol Head Neck Surg 1992;118:1023-7.

13. Walner DL, Stern Y, Gerber ME, Rudolph C, Baldwin CY, Cotton RT. Gastroesophageal reflux in patients with subglottic stenosis. Arch Otolaryngol Head Neck Surg 1998;124: 551-5.

14. Halstead LA. Gastroesophageal reflux: a critical factor in pediatric subglottic stenosis. Otolaryngol Head Neck Surg 1999;120:683-8.

15. Walner DL, Stern Y, Cotton RT. Margins of partial cricotracheal resection in children. Laryngoscope 1999;109:1607-10.

Ann Otol Rhinol Laryngol 110:2001

EFFECT OF IN VITRO IRRADIATION OF DONOR LARYNGES ON CYCLOSPORINE REQUIREMENTS AND REJECTION RATES IN RAT LARYNGEAL TRANSPLANTATION

STEVEN W. BARTHEL, MD
JONATHAN MYLES, MD
OLIVIA DAN
MARSHALL STROME, MD
CLEVELAND, OHIO

Total lymphoid irradiation is an acknowledged adjunctive immunosuppressant in whole organ transplantation in humans and animals. Local irradiation administered for a similar purpose is at best controversial. We evaluated in vitro donor larynx irradiation immediately preceding laryngeal transplantation as an immunomodulator. Each donor larynx was pretreated with 7.34 Gy of radiation in vitro. After transplantation, cyclosporine was administered in doses of 5 mg/kg per day, 2.5 mg/kg per day, and 1 mg/kg per day for trial lengths of 15 days and 30 days. Each of these 6 groups consisted of 10 rats per group. Earlier data have shown cyclosporine dosed at 5 mg/kg per day, without irradiation, administered for 1 month to have varied efficacy. Established histologic criteria were used to determine rejection patterns. All recipient rats survived the 15-day and 30-day trials. In all 10 rats receiving 5 mg/kg per day of cyclosporine for 15 days, the harvested transplanted larynges were viable without evidence of meaningful rejection (mild rejection). In 9 of the 10 rats receiving 5 mg/kg per day of cyclosporine for 30 days, the transplanted larynges displayed no meaningful rejection (mild rejection). In 9 of the 10 rats receiving 2.5 mg/kg per day of cyclosporine for 15 days, the transplanted larynges displayed no meaningful rejection (mild rejection). One rat receiving 2.5 mg/kg per day of cyclosporine for 15 days had a transplanted larynx that displayed moderate rejection. In all 10 rats receiving 2.5 mg/kg per day of cyclosporine for 30 days, the transplanted larynges displayed no meaningful rejection (mild rejection). At 15 days, 5 rats treated with 1 mg/kg per day of cyclosporine displayed mild rejection, 2 displayed moderate rejection, 2 displayed advanced to moderate rejection, and 1 displayed severe rejection. At 30 days, 4 rats treated with 1 mg/kg per day of cyclosporine displayed moderate rejection, 2 displayed advanced to moderate rejection, and 4 displayed severe rejection. We conclude that pretransplantation in vitro irradiation of donor larynges has immunomodulatory effects, allowing reduced cyclosporine immunosuppression with less rejection.

KEY WORDS — cyclosporine, irradiation, rejection, transplant.

INTRODUCTION

To date, organ transplantation has been lifesaving for patients with any of several diseases affecting visceral organs. However, applications of transplantation technology to cancer patients have been limited in general, and specifically for organs termed "nonvital." Current morbidity rates after laryngectomy make laryngeal transplantation ethically applicable for cancer patients if the risk of recurrence associated with chronic immunosuppression can be addressed.

Although accepted immunosuppressive regimens increase the risk for both new primary and recurrent tumors, the reasons for this increase remain poorly understood. The suppression of lymphocyte function, a requisite for allograft longevity, is presumably responsible. Yet unknown is whether modifications in total dose or peaks in accepted immunomodulatory protocols can affect the incidence of tumors.

Whereas total lymphoid irradiation is acknowledged as having a meaningful immunomodulatory effect on transplantation and systemic maladies, the effect of local donor irradiation has been variable. Studies of rat lung allograft transplantation have documented that local donor irradiation, before transplantation, allays the rejection response.[1] However, cellular, histologic, and dose-response data are lacking and require definition. Conversely, current data do not support donor irradiation for small intestine and liver transplantation in rats.[2,3] As yet, the efficacy of combined local pretransplantation in vitro donor irradiation and low-dose cyclosporine has not been evaluated in rat laryngeal allografts. This study was conducted to establish the efficacy of this combination in preventing rejection, thereby further defining the requirements for successful allograft transplantation in humans.

MATERIALS AND METHODS

Animal and Transplantation Technique. Lewis and LBNf-1 rats were used as recipients and donors, re-

---

From the Departments of Otolaryngology (Barthel, Dan, Strome) and Pathology (Myles), The Cleveland Clinic Foundation, Cleveland, Ohio.
This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of The Cleveland Clinic Foundation.

Presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, April 26-27, 1999.

CORRESPONDENCE — Marshall Strome, MD, Dept of Otolaryngology and Communicative Disorders, A71, Cleveland Clinic Foundation, 9500 Euclid Ave, Cleveland, OH 44195.

NUMBER OF ANIMALS WITH TRANSPLANTED LARYNGES PER REJECTION GRADE AT GIVEN DOSE OF CYCLOSPORINE FOR 15-DAY AND 30-DAY TRIAL PERIODS

	Histologic Grading of Rejection			
Cyclosporine Dose Per Day	*Mild*	*Moderate*	*Moderate to Advanced*	*Severe*
15-day trial				
5 mg/kg	10			
2.5 mg/kg	9	1		
1 mg/kg	5	2	2	1
30-day trial				
5 mg/kg*	9			
2.5 mg/kg	10			
1 mg/kg	4	2	4	

*One animal had anastomotic thrombosis.

spectively. All animals were humanely treated in accordance with the guidelines of the Animal Use Committee of the Cleveland Clinic Foundation and those described in the *Guide for the Care and Use of Laboratory Animals*, prepared by the National Academy of Sciences.

The transplantation technique used has been published,[4] but is briefly described here. Before larynx harvest, the donor vascular pedicles (the right and left common carotid, external carotid, and superior thyroid arteries) are isolated. The donor larynx is transplanted into a pocket in the recipient's left neck in tandem with the native larynx. The right common carotid artery of the donor is anastomosed end-to-side to the recipient's left common carotid artery. Venous drainage is accomplished by an end-to-side arteriovenous shunt, joining the donor's left common carotid artery to the recipient's external jugular vein.

Radiotherapy Technique. A 300-kV Siemens (Munich, Germany) orthovoltage unit with a standard thoracic filter was used. Harvested donor larynges bathed in preservation solution were positioned below the radiation port. Each larynx was treated in 1 fraction to a total of 7.34 Gy by means of a single vertical port via a 4 × 4-cm aperture at a vertical distance of 4 cm.

Trial Design. The trials lasted either 15 days or 30 days. Sixty Lewis rats received irradiated LBNf-1 allografts. All donor larynges received 7.34 Gy of radiation with transplantation following immediately. The rats received cyclosporin A dosed at 5 mg/kg per day, 2.5 mg/kg per day, or 1 mg/kg per day for 15 days or 30 days. Each treatment group comprised 10 rats. The 5–mg/kg per day cyclosporine dose was chosen on the basis of earlier data,[5] with arbitrary lower doses chosen as 2.5 mg/kg per day and 1 mg/kg per day. A historical control group was used, consisting of 6 laryngeal transplant recipient rats who received 5 mg/kg per day of cyclosporine alone, without in vitro irradiation. In this model, a cyclosporine dose of 5 mg/kg per day for 30 days had previously been determined by the senior author (M.S.) to be the dose at which rejection can be prevented for only a minority of transplanted larynges.[5]

End Points Assessed. Clinical viability was assessed through evaluation of vascular patency and observation of gross laryngeal architecture. Histologic studies using whole-organ sections were performed on all clinically viable transplanted larynges. Specific details pertaining to our viability criteria and histologic grading have been published.[6]

RESULTS

Of the 60 rats receiving laryngeal transplants, all lived for the 15-day and 30-day trial periods. The results are shown in the Table. In all 10 rats receiving 5 mg/kg per day of cyclosporine for 15 days, the harvested transplanted larynges were viable and without evidence of meaningful rejection (mild rejection). In 9 of the 10 rats receiving 5 mg/kg per day of cyclosporine for 30 days, the harvested transplanted larynges were viable and without evidence of meaningful rejection (mild rejection). One rat receiving 5 mg/kg per day of cyclosporine for 30 days had the transplanted larynx compromised by an arterial anastomotic thrombosis.

In 9 of the 10 rats receiving 2.5 mg/kg per day of cyclosporine for 15 days, the harvested transplanted larynges were viable and without evidence of meaningful rejection (mild rejection). One rat receiving 2.5 mg/kg per day of cyclosporine for 15 days had a transplanted larynx that displayed moderate rejection. In all 10 rats receiving 2.5 mg/kg per day of cyclosporine for 30 days, the harvested transplanted larynges were viable and without evidence of meaningful rejection (mild rejection).

At 15 days, 5 rats treated with 1 mg/kg per day of cyclosporine displayed mild rejection, 2 displayed moderate rejection, 2 displayed advanced to moderate rejection, and 1 displayed severe rejection. At 30 days, 4 rats treated with 1 mg/kg per day of cyclosporine displayed moderate rejection, 2 displayed advanced to moderate rejection, and 4 displayed severe rejection.

The histologic criteria evaluated included lymphocytic infiltration and preservation of minor salivary glands, muscle, lamina propria, surface epithelium, and brush border. Details of the histologic grading system used have been previously published.[6]

A comparison of histologic rejection between our

(P24740

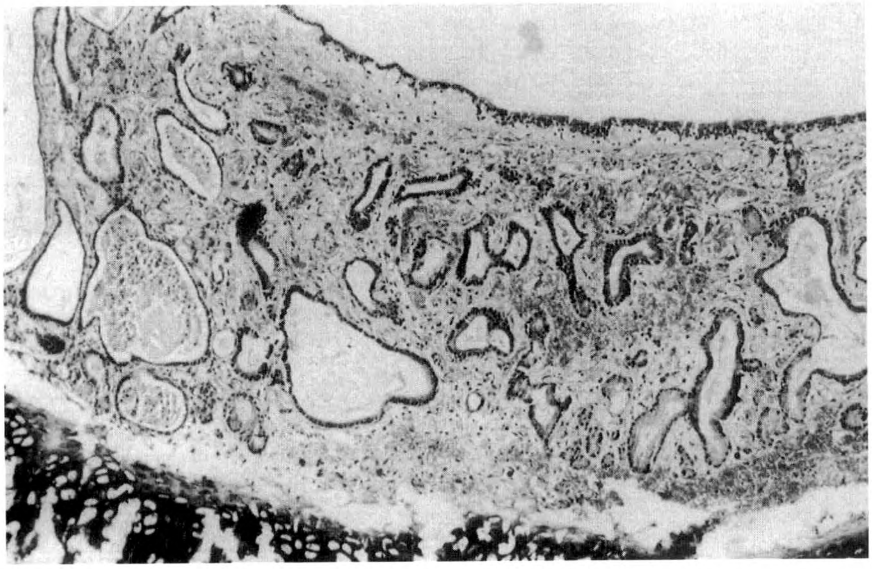


Fig 1. Mild rejection after daily doses of cyclosporine alone (5 mg/kg per day) for 30 days. Note intact cartilage (lower left) and architecture, intact ciliated surface respiratory epithelium with some lymphocytic infiltration, and residual minor salivary glands with irregular dilatation.

treatment groups (cyclosporine and pretransplantation in vitro irradiation) and our historic controls (cyclosporine alone at 5 mg/kg per day) is illustrated in Figs 1-5. After 30 days, with 5 mg/kg per day of cyclosporine alone, 2 transplanted larynges showed mild rejection (Fig 1), 2 showed moderate rejection, and 2 showed severe rejection (Fig 2). With pretransplantation in vitro irradiation and cyclosporine doses of 5 mg/kg per day (n = 10) or 2.5 mg/kg per day (n = 10) for 30 days, all nonthrombosed transplanted larynges (19/20) showed mild rejection only, with representative histologic findings seen in Figs 3 and 4, respectively. With pretransplantation in vitro irradiation and a very low cyclosporine dose of 1 mg/kg per day for 30 days, 4 of 10 larynges displayed only moderate rejection (Fig 5). When evaluated at 30 days, our treatment groups receiving in vitro irradiation and cyclosporine showed less rejection at cyclosporine doses of 5 mg/kg per day and 2.5 mg/kg per day than historic controls receiving 5 mg/kg per day of cyclosporine alone. Further, with in vitro irradiation and a very low dose of 1 mg/kg per day of cyclosporine, histologic rejection was only slightly greater than it was with 5 mg/kg per day of cyclosporine alone.

DISCUSSION

Currently, a presumed increased risk of recurrent cancer is a significant obstacle to overcome if laryngeal transplantation is to be considered in the presence of malignancy. In the single partial laryngeal transplantation performed, cancer recurrence led to death.[7] This risk may be reduced or obviated by varying therapeutic immunosuppression regimens in both dose and sequencing. Immunosuppressive protocols with relative sparing of CD8 T cells seem better able to prevent allograft rejection while limiting the associated risk of tumor recurrence. For example, studies comparing the incidence and severity of associated cutaneous squamous cell carcinoma in transplant

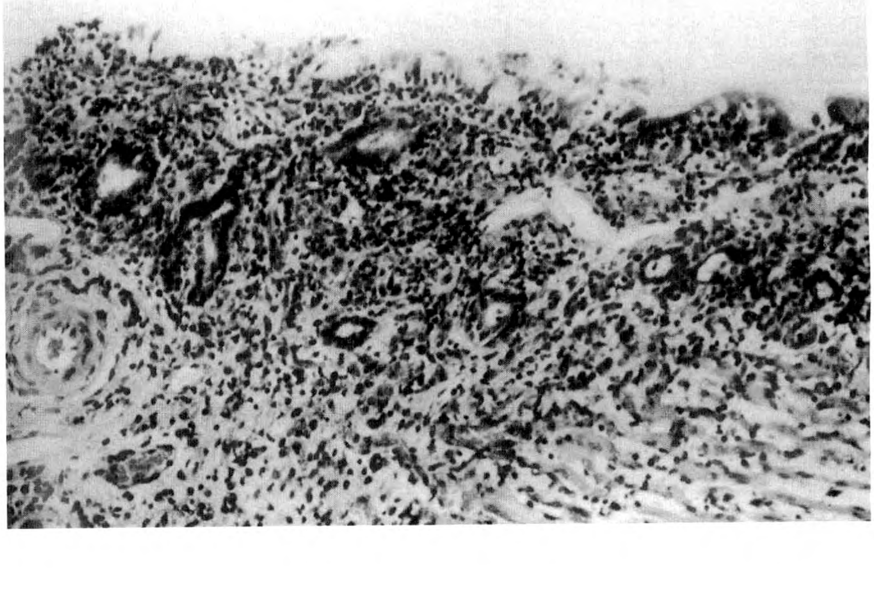


Fig 2. Severe rejection after daily doses of cyclosporine (5 mg/kg per day) for 30 days. Note loss of architecture, increased numbers of lymphocytes and histiocytes, adjacent artery with intimal proliferation, and absence of ciliated epithelium and minor salivary glands.

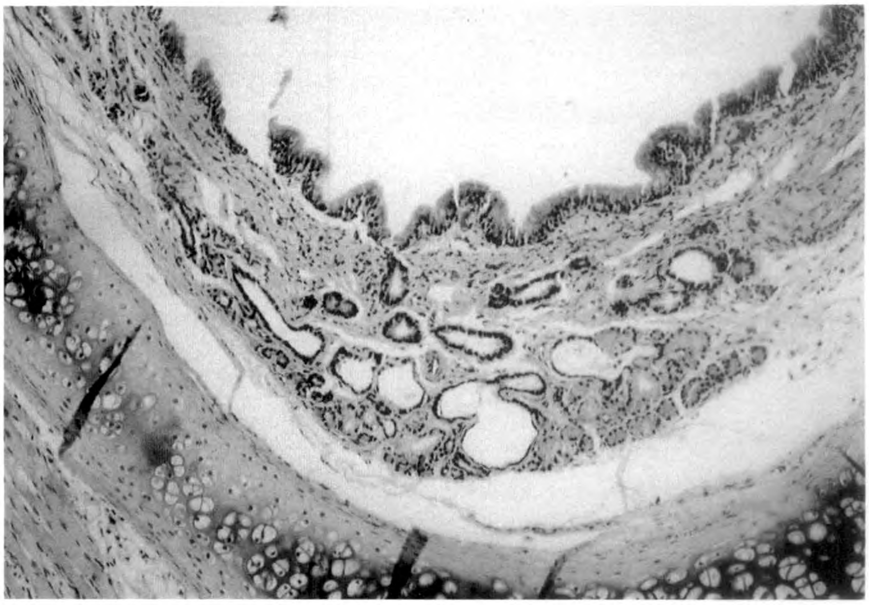


Fig 3. Mild rejection after pretransplantation in vitro irradiation and daily doses of cyclosporine (5 mg/kg per day) for 30 days. Note intact cartilage (lower border) and architecture, intact ciliated epithelium with some lymphocytic infiltration and sectioning artifact, and residual minor salivary glands with irregular dilatation.

recipients receiving azathioprine (indiscriminate cytotoxicity) and cyclosporine (selective immunoregulation) showed a decreased incidence of carcinoma and an improved survival rate with cyclosporine.[8]

Cyclosporine remains the gold standard in immunosuppression for organ transplantation. Modulation occurs in both the cellular and humoral responses via downregulation of the interleukin-2 receptor and a decrease in interleukin-2 production. Differing from earlier-generation immunosuppressants, it spares CD8 T cells.[9]

The efficacy of total lymphoid irradiation as an immunosuppressive regimen in organ transplantation has been established.[10] Less clear is the role of local irradiation and, specifically, donor irradiation. Improvements with preoperative irradiation of donor lung allografts may result from a decrease in peribronchial lymphatics.

This study design and implementation included the following premises based on an extensive prior experience with this surgical model and data interpolation. Our established rejection grading system affords ready delineation of histologic groupings, obviating the need for blinded reading. Because there was essentially no difference in the surgical technique, the senior author's published data on cyclosporine alone were considered very adequate to serve as the control group. A 30-day trial encompasses the period of early acute rejection most significant in clinical transplantation. The duration of our trial was chosen for this reason.

Significant in this study is the fact that at 30 days,

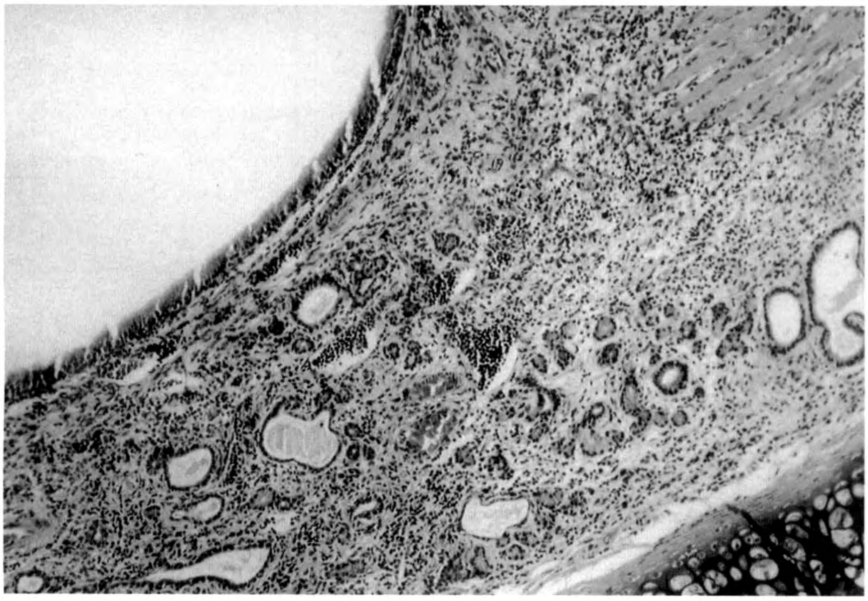


Fig 4. Mild rejection after pretransplantation in vitro irradiation and daily doses of cyclosporine (2.5 mg/kg per day) for 30 days. Note intact cartilage (lower right) and architecture, intact ciliated epithelium with some lymphocytic infiltration and sectioning artifact, and residual minor salivary glands with irregular dilatation.

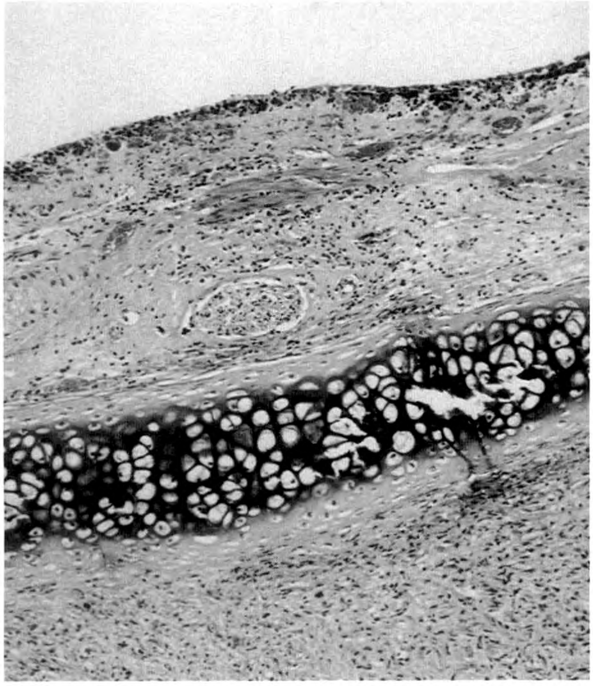


Fig 5. Moderate rejection after pretransplantation in vitro irradiation and daily doses of cyclosporine (1 mg/kg per day) for 30 days. Note intact cartilage, nonciliated epithelium with some lymphocytic infiltration, and lack of minor salivary glands.

at a relatively low dose of cyclosporine (2.5 mg/kg per day), with pretransplantation in vitro irradiation, all transplanted larynges (n = 10) showed essentially normal histologic findings (grade = mild rejection). In comparison, in our earlier study,[5] at a dose of 5 mg/kg per day of cyclosporine alone (n = 6), there was a markedly different outcome, with 2 subjects showing mild rejection, 2 moderate rejection, and 2 severe rejection. Further, at the same 30-day interval, the 1–mg/kg per day group with in vitro irradiation showed only slightly more histologic rejection than the 5–mg/kg per day cyclosporine-alone group.

These results suggest that the lowest effective adjunctive dose of cyclosporine is 2.5 mg/kg per day if transplantation is preceded by in vitro irradiation of the donor larynx. However, in combination with mycophenolate mofetil and/or steroids, further reduction of cyclosporine dosing could be possible. Utilizing in vitro irradiation has allowed the use of low doses of cyclosporine. Future studies should evaluate the risk of recurrent cancer at low levels of immunosuppression to allow consideration of laryngeal transplantation after extirpation for malignancy. This would allow organ preservation via laryngeal transplantation for advanced and recurrent laryngeal cancer without compromising oncological principles.

CONCLUSION

Pretransplantation in vitro donor larynx irradiation appears to lower the cyclosporine dose required to prevent rejection of transplanted rat larynges. These findings are an improvement on those previously published for 5 mg/kg per day of cyclosporine alone.

REFERENCES

1. Prop J, Nieuwenhuis P, Wildervuur C. Lung allograft rejection in the rat. Transplantation 1985;40:25-30.

2. Lee TK, Langrehr JM, Moynihan HL, Stangl MJ, Lee KK, Schraut WH. Failure of donor irradiation to prolong allograft survival in small bowel transplantation in rats. Transplant Proc 1992;24:1183-4.

3. Sun J, McCaughan GW, Gallagher ND, Sheil AG, Bishop GA. Deletion of spontaneous rat liver allograft acceptance by donor irradiation. Transplantation 1995;60:233-6.

4. Strome S, Sloman-Moll E, Samonte BR, Wu J, Strome M. Rat model for a vascularized laryngeal allograft. Ann Otol Rhinol Laryngol 1992;101:950-3.

5. Strome M, Strome S, Darrell J, Wu J, Brodsky G. The effects of cyclosporin A on transplanted rat allografts. Laryngoscope 1993;103:394-8.

6. Strome S, Brodsky G, Darrell J, Wu J, Strome M. Histopathologic correlates of acute laryngeal allograft rejection in a rat model. Ann Otol Rhinol Laryngol 1992;101:156-60.

7. Kluyskens P, Ringoir S. Follow-up of a human larynx transplantation. Laryngoscope 1970;80:1244-50.

8. Hardie IR. Skin cancer in transplant recipients. Transplant Rev 1995;9:1-16.

9. Kupiec-Weglinski JW, Filho MA, Strom TB, Tilney NL. Sparing of suppressor cells: a critical action of cyclosporine. Transplantation 1984;38:97-101.

10. Strober S. Total lymphoid irradiation in alloimmunity and autoimmunity. J Pediatr 1987;111:1051-5.

Ann Otol Rhinol Laryngol 110:2001

EFFECT OF MITOMYCIN-C ON VOCAL FOLD HEALING IN A CANINE MODEL

C. GAELYN GARRETT, MD
JOSEPH SOTO, MD
JOHN RIDDICK
CHERYL R. BILLANTE, PHD
LOU REINISCH, PHD
NASHVILLE, TENNESSEE

For evaluation of the effect of topical mitomycin-C on vocal fold healing and return of function after surgical excision of mucosa, 6 dogs underwent suspension microlaryngoscopy with bilateral microflap excision of vocal fold mucosa. Topical mitomycin-C, a chemotherapeutic agent and a fibroblast inhibitor, was applied randomly to one side, with the contralateral side serving as the control. Laryngeal videostroboscopy (LVS) was performed on each animal before the operation, at 2 weeks, and before sacrifice at 4 weeks. Both functional analysis with LVS and histologic analysis were performed in a blinded fashion to determine the effect of mitomycin-C on healing. Histologic analysis was augmented with computer morphometrics. The LVS revealed diminished mucosal wave vibration in the vocal folds treated with mitomycin-C, as well as a more atrophic appearance to the vibratory surface. The vocal folds treated with mitomycin-C showed fewer fibroblasts and less collagen within the superficial layer of the lamina propria than the control vocal folds ($p < .05$, Student's *t*-test, paired analysis). Inflammatory infiltrate was not significantly different between the two sides. Consistent with the known suppression of fibroblast proliferation by mitomycin-C, the treated vocal folds showed less connective tissue response to the surgical injury. In contrast to the reported positive effects of mitomycin-C on tracheal and glottic stenosis, the observed decrease in the healing response in the present study had negative consequences on the vocal fold vibratory pattern.

KEY WORDS — mitomycin-C, vocal fold, wound healing.

INTRODUCTION

Current phonosurgery techniques are based on a recently improved understanding of vocal fold mechanics and vocal fold pathology. With Hirano's landmark description of the cover-body theory of vocal fold vibration and our increasing understanding of the histopathology of benign vocal fold lesions, laryngologists have sought to optimize voice outcome with respect to surgical techniques.[1,2] Microflap techniques allow dissection and excision of the cyst, polyp, or mature nodule within the superficial layer of the lamina propria, while preserving the normal overlying mucosa and underlying vocal ligament.[3-5] The goal of these techniques is to minimize scar formation and subsequent tethering of the mucosal cover. These techniques have been shown to result in both subjective and objective improvements in vocal fold vibration and voice quality. However, the inevitable result of any surgical excision is some amount of scar formation.

Adjunctive therapy has been investigated to help modulate the wound healing process. Steroids, both systemic and topical, have been used clinically as an empiric treatment to reduce scar formation after laryngeal surgery. A clinical study by Bouchayer and Cornut[6] reported on improved vocal fold suppleness, closure, and voice quality following injection of steroids into the vocal fold immediately prior to elevating a microflap. Coleman et al,[7] however, noted no differences in vocal fold function as measured by laryngeal videostroboscopy (LVS) in a study in which steroids were placed within the microflap pocket of a dog model. They did note that the wound healing response was delayed but not lessened in the steroid group.

Mitomycin-C (MMC) is an antimetabolite that has been shown to inhibit fibroblast proliferation when applied topically. This property has led to its common use in ophthalmology procedures to reduce scar formation. More recently, MMC was shown experimentally to successfully augment the surgical treatment of subglottic stenosis and posterior glottic stenosis.[8-10] In these studies, the abnormal wound healing and cicatrix (stenosis) are modulated to the return of a smooth mucosal surface by means of topical MMC.

Since scarring of the lamina propria may diminish the voice outcome from vocal fold surgery, reduc-

---

From the Department of Otolaryngology, Vanderbilt Bill Wilkerson Center for Otolaryngology and Communication Sciences, Vanderbilt University Medical Center, Nashville, Tennessee. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Vanderbilt University.

Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

CORRESPONDENCE — C. Gaelyn Garrett, MD, Dept of Otolaryngology, Vanderbilt Voice Center, 1500 21st Ave S, Suite 2700, Nashville, TN 37212.

tion or prevention of postoperative scar formation during the healing phase would be beneficial. This study examined the effects of topical MMC on vocal fold healing following microflap excision of mucosa in a dog model. Both functional and histologic parameters were examined.

MATERIALS AND METHODS

All procedures and actions were approved and monitored by the institutional animal care and use committee. Six mongrel dogs underwent suspension microlaryngoscopy. Each animal underwent 3 procedures. The initial procedure consisted of preoperative LVS followed by direct microlaryngoscopy with bilateral microflap excision of vocal fold mucosa. One vocal fold in each dog was topically treated with MMC, and the opposite side served as the control. The second and third procedures consisted of suspension LVS at 2 weeks and 4 weeks after surgical excision. The animals were painlessly sacrificed after the final LVS was performed, and the larynges were harvested for histologic examination.

For each procedure, the animal was placed supine on an operating table and anesthetized with intramuscular agents (telazol 4 mg/kg, xylazine hydrochloride 0.275 mg/kg) and allowed to breathe spontaneously. The intraoperative monitoring consisted of an electrocardiogram and measurements of the depth and quantity of spontaneous respiration and the level of pain threshold. Each animal received preoperative and postoperative antibiotics (penicillin G procaine and gentamicin sulfate). Postoperative pain was managed with flunixin meglumine.

Suspension microlaryngoscopy was performed with a modified Ossoff-Karlen laryngoscope (Pilling, Fort Washington, Pa) with a longer lip to accommodate the different canine laryngeal anatomy. A 0° rigid telescope (Storz model 8701A, St Louis, Mo) was positioned 2 cm above the glottic inlet. The LVS equipment included a Bruel & Kjaer stroboscopy unit (type 4914, Naerum, Denmark), a Panasonic SVHS recorder (model AG-7350, Matsushita Communication Industrial Co, Osaka, Japan), and a Toshiba microchip camera (Toshiba model IK-C40A, New York, New York). Airflow was delivered from an air compressor through a 14-gauge needle inserted transcutaneously into the trachea at approximately the fifth interspace. The compressor was set at a constant pressure for all stroboscopic examinations. The amount of airflow used was the minimum flow needed to trigger vocal fold vibration and phonation. This flow rate was kept constant and was consistent for all animals. Vocal fold adduction was achieved with external manual pressure on the posterior larynx.

Once adequate LVS data had been acquired at the initial procedure, the animal was prepared for endoscopic laryngeal surgery. With the animal still under suspension laryngoscopy, an operating microscope (model OPMI-DFC, Carl Zeiss, Inc, Thornwood, Pa) with a 400-mm lens was positioned for a magnified view of the larynx. A medial microflap incision was made near the medial edge along the middle third of each membranous vocal fold. A surgical plane was developed with elevators within the superficial aspect of the lamina propria. An ellipse of the elevated mucosal cover was excised, resulting in an area of exposed deeper lamina propria (Fig 1). All microflap excisions were performed by the first author, and care was taken to have a consistent depth of microflap elevation. The same excision was performed on both vocal folds of each animal. Mitomycin-C (0.4 mg/mL) was then applied topically to one side, chosen randomly, for 3 minutes with a cottonoid pledget. Care was taken to avoid contact of the MMC with the opposite vocal fold. The treated vocal fold was then cleansed with saline solution to avoid exposure of the untreated control vocal fold to residual MMC. The concentration of MMC and the single topical treatment are consistent with studies in which MMC was used to treat subglottic stenosis and are consistent with the concentration and duration of application used in clinical ophthalmology.

Repeat LVS was performed in all animals 2 weeks and 4 weeks after the initial microflap procedure by means of the same technique described above. Immediately after the 4-week LVS and with the animal still under anesthesia, the animals were painlessly sacrificed with a lethal dose of a barbiturate. The larynges were harvested and kept in 10% formalin for several days before sectioning. Coronal sections at the midfold level were processed in the medical center's division of histopathology.

Histology. The specimens were decalcified, sectioned by a rotary microtome (4 to 5 µm), and stained by standard techniques. Each section was stained with hematoxylin and eosin (H & E) and Masson's trichrome. To aid in standardization, all staining was performed by the same senior technician. The H & E sections were used in the analysis of the inflammatory infiltrate. The trichrome stain was used in the analysis of the connective tissue components.

Computer morphometric analysis was performed on each vocal fold section. The computer processing system consisted of a Zeiss microscope system (Zeiss model 4521-85, Jena, Germany) and a digital image capturing program for the computer. Each vocal fold was examined in a blinded fashion for the area of the microflap excision. Once located, the area was captured with the 2.5×, 10×, and 40× objectives and saved

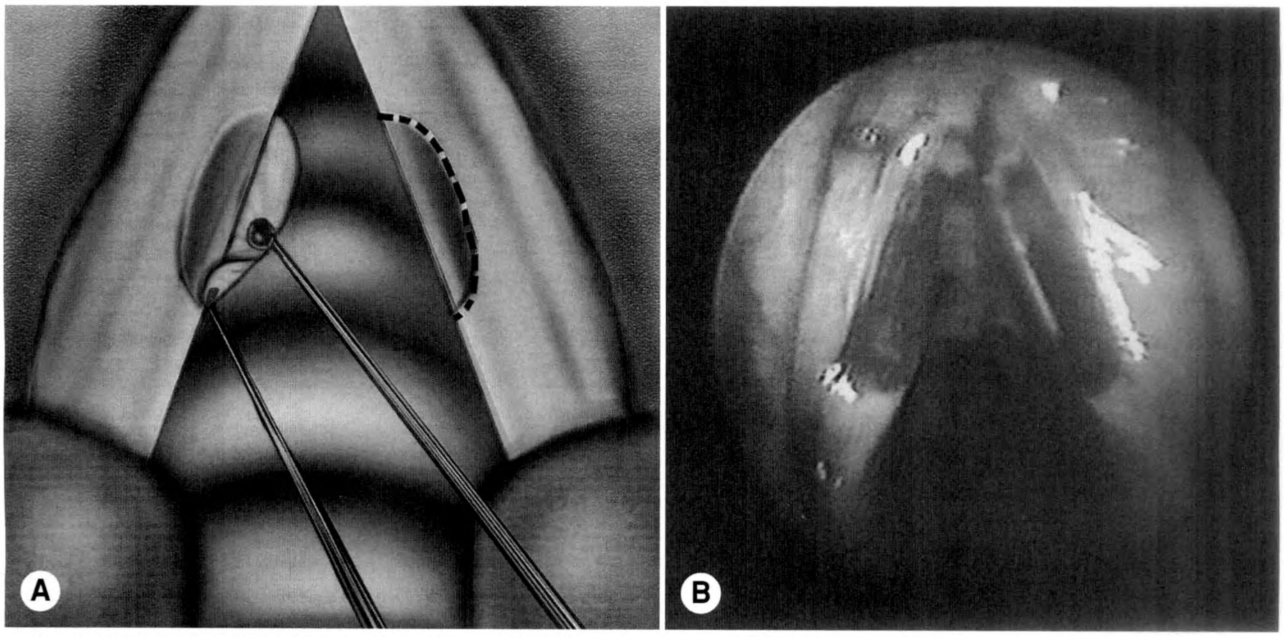


Fig 1. A) Sketch shows approximate area and location of mucosa removed by microflap elevation technique from each vocal fold. B) Intraoperative photograph shows areas of excision.

as TIFF images. The images were then analyzed with an image processing software program (Adobe Photoshop 5, San Jose, Calif). The trichrome stains fibroblasts a deep pink, and collagen blue. With Photoshop, the fibroblasts within the selected area of the excision were counted by identifying the color range and cell morphology specific to the fibroblasts. The inflammatory lymphocytic infiltrate was measured on the H & E sections by means of the same technique used for the fibroblasts. The amount of collagen was determined by first specifying and measuring the area of interest. In order to more clearly highlight the color differences of the image, the image was equalized digitally. The color range specific for the collagen (blue) was selected. The computer program then calculated the number of pixels within the specified color range. The ratio of the number of pixels to the measured area of interest was calculated. Statistical analyses of the fibroblasts, inflammatory infiltrate, and collagen concentrations were performed with a 2-tailed Student's *t*-test with paired comparison. Statistical significance was defined as $p < .05$.

Laryngeal Videostroboscopy Analysis. All LVS samples were condensed with a video editor into a single tape of approximately 45-second samples. Each animal had 3 LVS samples (preoperative, 2 weeks, 4 weeks). The animals were randomly assigned a number so the evaluators would be blinded to the treated side. For each animal, the 3 samples were placed in random order, but grouped together on the tape. The evaluators were told that the 3 grouped samples were from the same animal, but at randomly ordered time periods. Five speech and language pathologists, all trained in the acquisition and interpretation of human LVS, qualitatively evaluated the 18 samples. A standard form used in our clinical practice was also used for this study. Vocal fold anatomy and LVS parameters (mucosal wave, amplitude) were described. The mucosal wave analysis examined each vocal fold individually and rated the wave as normal, great, decreased, abnormal pattern, or absent. Vocal fold anatomy was examined for edema, bowing, inflammation, and excrescence. The subjective analyses were then collated to produce trends.

RESULTS

All 6 dogs survived the 4 weeks of the study. No complications were encountered.

Histology. Computer morphometric analysis provided data on the concentrations of fibroblasts, inflammatory cells, and collagen within the area of the microflap excisions. In comparison with the untreated control vocal folds, the MMC-treated vocal folds had fewer fibroblasts ($p < .05$) and less collagen within the lamina propria ($p < .05$; Fig 2). There was no statistically significant difference in inflammatory infiltrate (see Table).

Laryngeal Videostroboscopy. The responses from the blinded evaluators were collated and analyzed on the basis of anatomic characteristics (edema, inflammation, excrescence, bowing) and LVS findings (symmetry, amplitude, and mucosal wave). Comparisons were made at the time of the initial procedure, at 2 weeks, and at 4 weeks. There were no significant differences in the anatomic or LVS characteristics noted between the vocal folds of each animal at the preop-

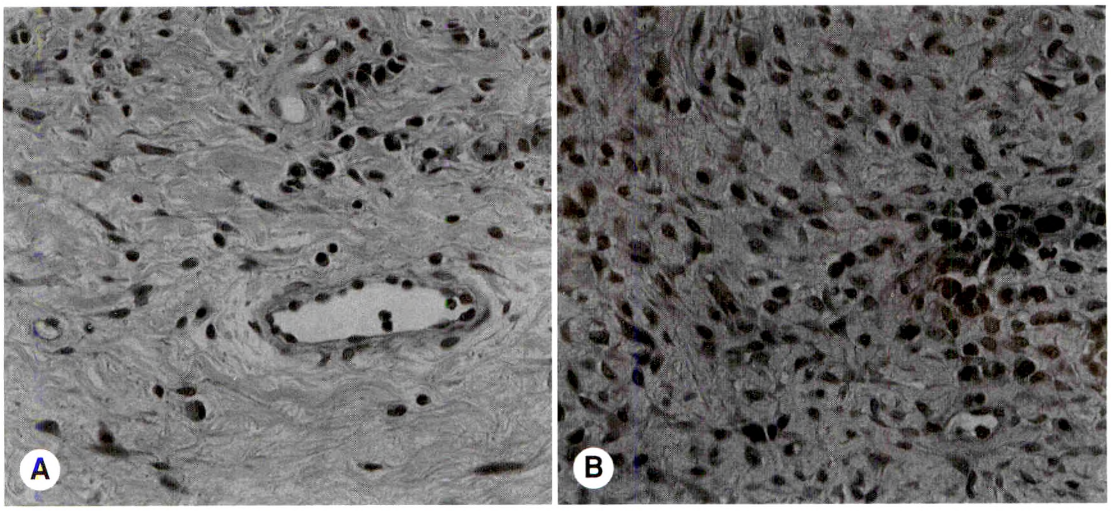


Fig 2. Lamina propria from dog 1 (H & E, original ×40 on objective lens). A) From vocal fold treated with mitomycin-C. B) Control. Increase in number of fibroblasts (dark spindle-shaped cells) is clear.

erative evaluation. At 2 weeks, the MMC-treated vocal folds in 2 animals had a decreased mucosal wave as compared with the control side. The other 4 animals showed similar decreased or adynamic segments at the excision site in both the MMC and control vocal folds. No significant anatomic differences were observed at 2 weeks, with both sides usually showing mild to moderate edema and inflammation with evidence of a concavity at the excision site (Fig 3). At 4 weeks, the MMC-treated vocal folds in 3 of the 6 dogs either had a persistent concavity at the excision site or had generalized atrophy as compared to the control side. This corresponded to a decreased mucosal wave in the treated vocal folds of these 3 dogs as compared to the control vocal folds. The other 3 dogs had no significant differences in anatomic appearance or LVS characteristics between the MMC-treated and control vocal folds. In summary, none of the MMC-treated vocal folds had an improved anatomic appearance or improved stroboscopic findings at 2 or 4 weeks after the procedure.

DISCUSSION

Phonation depends upon the normal vibration of the true vocal folds on adduction. Any aberrancy of the smooth medial vibrating edge of the vocal folds or the layers of the lamina propria can alter the vibratory pattern of the vocal folds and lead to dysphonia. Benign non-neoplastic lesions of the vocal fold lamina propria such as polyps and cysts cause dysphonia by affecting vocal fold closure and/or the mucosal wave. Unfortunately, surgical excision of these lesions may also result in dysphonia, especially if the normal overlying mucosal cover is damaged or removed with stripping techniques. Both clinical and research experience has confirmed that fixation of the mucosal cover to the vocal ligament or vocalis muscle impairs vocal quality following vocal fold surgery. Woo et al[11] found that vocal fold scarring was the most common cause of postoperative dysphonia in a group of 62 patients undergoing surgery. Vocal fold stripping in a cat model resulted in stiffening of the mucosa and poor wound healing.[12] Despite significant advancements in vocal fold phonosurgery with the advent of microflap preservation surgery, scar formation remains the end product of the normal wound healing process.

Mitomycin-C has been shown to reduce scar formation in a variety of clinical settings. Its use has been most described in the ophthalmology literature, in terms of enhancement of the surgical treatment of glaucoma and pterygia formation.[13-15] Recently, the clinical use of MMC was reported in the otolaryngology literature as an adjunct in endolymphatic sac sur-

NUMBERS OF INFLAMMATORY CELLS AND FIBROBLASTS OBSERVED IN FIELD OF VIEW FROM MICROGRAPH, AND FRACTION OF OBSERVED AREA IDENTIFIED AS COLLAGEN

		Dog							
		1	*2*	*3*	*4*	*5*	*6*	*Mean Difference*	*p*
Inflammatory cells	Mitomycin-C	110	69	51	125	165	83		
	Control	323	63	128	125	38	54	−21	.67
Fibroblasts	Mitomycin-C	122	66	61	26	75	30		
	Control	557	127	250	172	110	299	−189	.026
Collagen	Mitomycin-C	0.195	0.284	0.237	0.280	0.396	0.310		
	Control	0.273	0.411	0.520	0.435	0.694	0.415	−0.175	.006

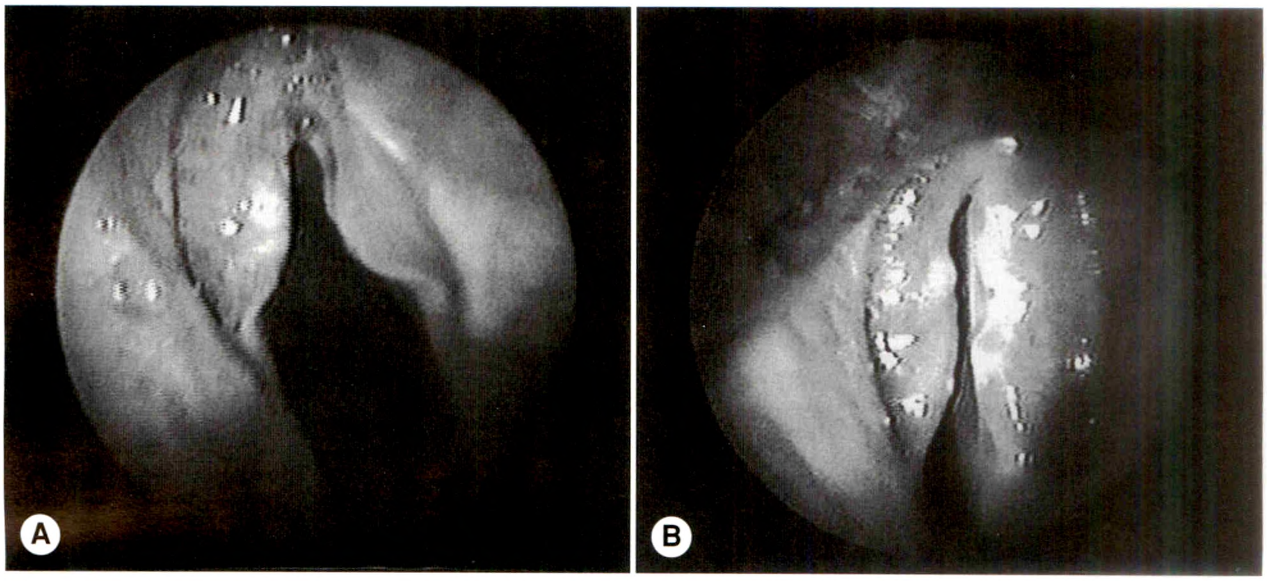


Fig 3. A) Bilateral tissue deficits give bowed appearance at 2 weeks. B) Persistent tissue deficit on mitomycin-C–treated left vocal fold at 4 weeks.

gery and sinus surgery.[16,17] Mitomycin-C is an antimetabolite isolated from the broth of *Streptomyces caespitosus*. Its primary use is as a chemotherapeutic agent in the treatment of certain gastrointestinal and genitourinary malignancies. Mitomycin-C selectively inhibits DNA synthesis. At higher concentrations, it also suppresses cellular RNA and protein synthesis. Topically, it has been shown to inhibit fibroblast proliferation; hence its clinical use as a modulator of the wound healing response. The major toxicity from MMC given systemically has been myelosuppression that can be life-threatening. In the ophthalmology literature, local complications have included scleral and corneal ulceration, scleromalacia, calcification, iritis, corneal perforation, and ocular hypotonia.[18,19] No systemic complications have been reported with topical use.

This study reports on the effects of topical MMC on exposed lamina propria in a canine vocal fold model. The operation performed was the equivalent of vocal fold stripping along the medial edge. Our goal was to ascertain whether MMC would reduce fibrosis in an area in which development of scar tissue can lead to significant postoperative complications, specifically, permanent dysphonia. We did not perform conventional microflap surgery with preservation of the mucosal cover, as the effect of MMC on wound healing might not be as easily appreciated with a lesser injury.

The study yielded both expected and unexpected results. As MMC has had beneficial effects on the wound healing response in the endoscopic treatment of glottic and subglottic stenosis, we anticipated a similar beneficial effect in vocal fold surgery. However, those vocal folds treated with MMC appeared to be more atrophic or to have a deficiency of the lamina propria as compared to the untreated vocal folds. This gross appearance was consistent with the histologic findings of decreased connective tissue components within the lamina propria. The fibroblasts of the vocal fold lamina propria produce more elastin than skin fibroblasts.[20] It is possible that the MMC not only modulated the wound healing process, but also affected the normal fibroblast products within the lamina propria, leading to adverse effects on the normal vocal fold vibratory pattern.

Since the completion of this study, we have begun an institutional review board–approved clinical study to evaluate the use of MMC as an adjunct in the surgical treatment of tracheal, subglottic, and glottic stenosis. Preliminary observations have shown that in some patients the effects of the MMC are delayed, with further reduction of cicatricial tissue seen beyond the 4-week duration of the present animal study. It is therefore possible that the atrophy of MMC-treated vocal folds might be even more pronounced with a longer duration of observation.

CONCLUSION

Consistent with the known suppression of fibroblast proliferation by MMC, the treated vocal folds showed less connective tissue response to the surgical injury. In contrast to the reported positive effects of MMC on tracheal and glottic stenosis, the observed decrease in the healing response in this present study had negative consequences on the vocal fold vibratory pattern by affecting the connective tissue components of the lamina propria.

REFERENCES

1. Hirano M. Surgical anatomy and physiology of the vocal fold. In: Gould WJ, Sataloff RT, Spiegel JR, eds. Voice surgery. Chicago, Ill: Mosby–Year Book, 1993:125-58.

2. Courey MS, Shohet JA, Scott MA, Ossoff RH. Immunohistochemical characterization of benign laryngeal lesions. Ann Otol Rhinol Laryngol 1996;105:525-31.

3. Courey MS, Gardner GM, Stone RE, Ossoff RH. Endoscopic vocal fold microflap: a three-year experience. Ann Otol Rhinol Laryngol 1995;104:267-73.

4. Sataloff RT, Spiegel JR, Heuer RJ, et al. Laryngeal minimicroflap: a new technique and reassessment of the microflap saga. J Voice 1995;9:198-204.

5. Courey MS, Garrett CG, Ossoff RH. Medial microflap excision of benign vocal fold lesions. Laryngoscope 1997;107:340-4.

6. Bouchayer M, Cornut G. Microsurgery for benign lesions of the vocal folds. Ear Nose Throat J 1988;67:446-66.

7. Coleman JR Jr, Smith S, Reinisch L, et al. Histomorphometric and laryngeal viseostroboscopic analysis of the effects of corticosteroids on microflap healing in the dog larynx. Ann Otol Rhinol Laryngol 1999;108:119-27.

8. Eliashar R, Eliachar I, Esclamado R, Gramlich T, Strome M. Can topical mitomycin prevent laryngotracheal stenosis? Laryngoscope 1999;109:1594-600.

9. Correa AJ, Reinisch L, Sanders DL, et al. Inhibition of subglottic stenosis with mitomycin-C in the canine model. Ann Otol Rhinol Laryngol 1999;108:1053-60.

10. Spector JE, Werkhaven JA, Spector NC, et al. Preservation of function and histologic appearance in the injured glottis with topical mitomycin-C. Laryngoscope 1999;109:1125-9.

11. Woo P, Casper J, Colton R, Brewer D. Diagnosis and treatment of persistent dysphonia after laryngeal surgery: a retrospective analysis of 62 patients. Laryngoscope 1994;104:1084-91.

12. Leonard RJ, Kiener D, Charpied G, Kelly A. Effects of repeated stripping on vocal fold mucosa in cats. Ann Otol Rhinol Laryngol 1985;94:258-62.

13. Kunitomo N, Mori S. Studies on the pterygium. Part IV. A treatment of the pterygium by mitomycin-C instillation. Nippon Ganka Gakkai Zasshi 1963;67:601-7.

14. Chen CW. Enhanced intraocular pressure controlling effectiveness of trabeculectomy by local application of mitomycin-C. Trans Asia-Pacific Acad Ophthalmol 1983;9:172-82.

15. Singh G, Wilson MR, Foster CS. Mitomycin eye drops as treatment for pterygium. Ophthalmology 1988;95:813-21.

16. Yazawa Y, Suzuki M, Kitano H, Kitajima K. Intraoperative mitomycin-C in endolymphatic sac surgery for Meniere's disease: a pilot study. ORL J Otorhinolaryngol Relat Spec 1999;61:188-94.

17. Ingrams DR, Volk MS, Biesman BS, Pankratov MM, Shapshay SM. Sinus surgery: does mitomycin-C reduce stenosis? Laryngoscope 1998;108:883-6.

18. Rubinfeld RS, Pfister RR, Stein RM, et al. Serious complications of topical mitomycin-C after pterygium surgery. Ophthalmology 1992;99:1647-54.

19. Schraermeyer U, Diestelhorst M, Bieker A, et al. Morphologic proof of the toxicity of mitomycin-C on the ciliary body in relation to different application methods. Graefes Arch Clin Exp Ophthalmol 1999;237:593-600.

20. Broadley C, Gonzalez DA, Nair R, Koriwchak MJ, Ossoff RH, Davidson JM. A tissue-culture model for the study of canine vocal fold fibroblasts. Laryngoscope 1995;105:23-7.

≈

NINTH INTERNATIONAL FACIAL NERVE SYMPOSIUM

The Ninth International Facial Nerve Symposium will be held July 29 to August 1, 2001, in San Francisco, California. For information, contact Office of Continuing Medical Education, University of California, San Francisco; telephone (415) 476-5808; fax (415) 502-1795; e-mail: info@ocme.ucsf.edu.

Ann Otol Rhinol Laryngol 110:2001

INTRAOPERATIVE ASSESSMENT OF STAPES MOVEMENT

ALEXANDER HUBER, MD
THOMAS LINDER, MD
MATTIA FERRAZZINI, MS
STEPHAN SCHMID, MD
NORBERT DILLIER, PHD
SANDRO STOECKLI, MD
UGO FISCH, MD
ZURICH, SWITZERLAND

A method is described that allows, for the first time, intraoperative vibration modes assessment of the acoustically stimulated stapes by means of scanning laser Doppler interferometry (LDI). The study was designed to answer the following questions: 1) Is LDI practical for taking measurements during surgery? 2) Are the results comparable to the findings in temporal bone preparations? and 3) Do the vibration characteristics of the stapes change after the posterior incudal ligament is detached from the incus? Seven patients with profound bilateral hearing loss who were undergoing cochlear implantation were included in the study. The measurement system was easily applicable for intraoperative measurements and allowed contact-free analysis with very high accuracy. No major differences in the results from the live human subjects and temporal bone preparations were observed. The stapes movement was predominantly pistonlike at the lower frequencies and became complex at higher frequencies. Sacrificing the posterior incudal ligament had no statistically significant effect on stapes vibration.

KEY WORDS — laser Doppler interferometry, middle ear mechanics, posterior incudal ligament.

INTRODUCTION

Middle ear vibrations in response to physiological acoustic stimulation are in the micrometer and nanometer range of displacement and are therefore difficult to assess. During the 20th century, methods for analysis of these vibrations became more and more sophisticated and finally allowed contact-free measurements with very high accuracy. At the present time, laser Doppler interferometry (LDI) appears to be the most reliable method. Although LDI is frequently employed for research in animals, temporal bone (TB) preparations, and even tympanic membrane evaluation of human subjects, so far it has not been available for direct middle ear measurements in live humans during surgery. Without LDI, the surgeon uses an instrument to wiggle the ossicles under visual control with an operating microscope and qualitatively estimates the mobility of the ossicular chain. The goal of this research was to quantitatively measure the ossicular chain vibration during surgery.

The movement of the stapes in response to physiological sound has been described as a rotation around a fixed vertical axis running through the posterior edge of the footplate in human TBs.[1] On the other hand, a purely pistonlike movement was described in another study of human TBs[2] and in anesthetized cats.[3] Further measurements in human TBs[4] indicated that both a rocking motion and a superimposed pistonlike movement are present. Recent studies in human TBs[5] and anesthetized cats[6] confirmed these findings. So far, no measurements of the stapes have been made in live human subjects, and it was the intention of the present study to focus on stapes measurements and compare the results to the findings in TB preparations.

In certain otologic operations, the posterior incudal ligament (PIL) has to be sacrificed to gain wider access to the middle ear. Although its acoustic function has been examined,[1] its importance for proper sound transmission is not known. It was a further goal of the present study to gain more information on the acoustic function of the PIL. The study was designed to answer the following questions: 1) Is LDI practical for taking measurements during surgery? 2) Are the results comparable to the findings in TB preparations? and 3) Do the vibration characteristics of the stapes change after the PIL is detached from the incus?

MATERIALS AND METHODS

Patient Selection. The study included 7 patients with profound bilateral hearing loss who were undergoing cochlear implantation. Before surgery, all patients (or their parents) were precisely informed and consented to participate in the study. The mean age was 31 years (range, 3 to 69 years).

Measurement Setup. A measuring system was developed based on a scanning laser Doppler interferometer (Polytec GmbH, Waldbronn, Germany). The sensor head and the scanning unit were suspended from a standard operating microscope stand (Contraves, Zurich, Switzerland). A built-in camera and a

---

From the Department of Otolaryngology–Head and Neck Surgery, University Hospital of Zurich, Zurich, Switzerland.
CORRESPONDENCE — Alexander Huber, MD, ENT Department, University Hospital of Zurich, CH-8091 Zurich, Switzerland.

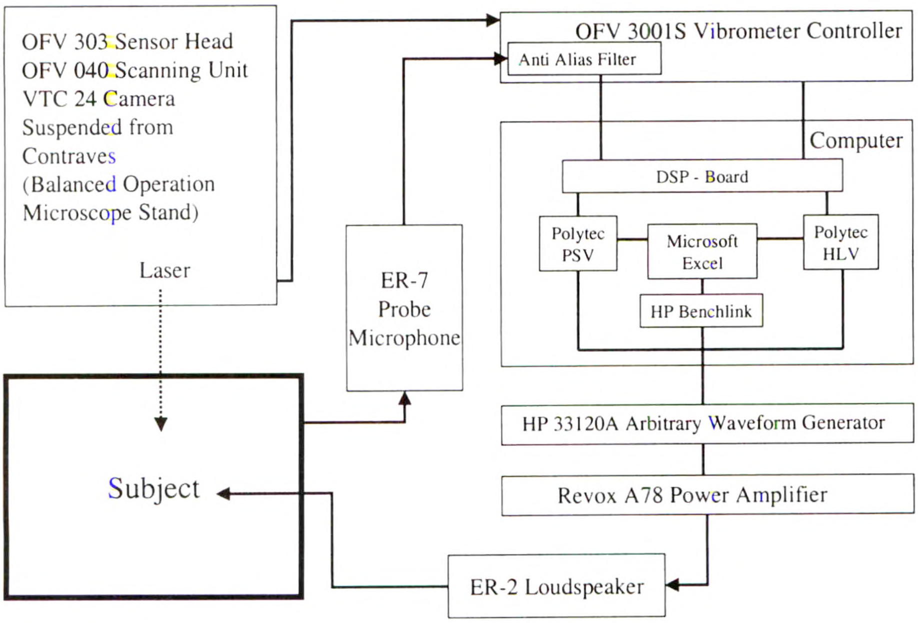


Fig 1. Measurement setup.

mirror system controlled the laser position on the object (Fig 1). A loudspeaker (ER-2, Etymotic Research, Elk Grove, Ill) placed in the external auditory canal produced the acoustic stimulus, which was controlled by a probe microphone (ER-7, Etymotic Research) close to the tympanic membrane. A multisine tone was used for stimulation. The signal contained 31 frequencies between 500 and 8,000 Hz that were produced by a signal generator (HP-33120A, Hewlett Packard, Palo Alto, Calif) and a power amplifier (Revox A78, Zurich, Switzerland). The sound pressure level (SPL) was calibrated by computer software (Polytec HLV 1.01, HP 34811A Benchlink, Microsoft Excel) to be 80 dB SPL for each stimulation frequency, resulting in a total sound pressure of 94 dB. The LDI software (Polytec PSV 200) allowed convenient analysis of the recorded data for each single frequency of the multisine tone. It yielded 3-dimensional animation and an iso-amplitude line view of the measured area. These functions were used to qualitatively assess the vibration mode of the stapes (rotatory versus translatory and rigid body versus bending). The data were also exported to other computer programs for further statistical analysis. Coherence is a measure of the noise level of an LDI measurement and was computed by the PSV software. For the analysis, only results with a coherence of 90% or better were used; this represents a signal-to-noise ratio of typically 10 to 25 dB.

Experimental Methods. The sterile probe microphone and loudspeaker were placed in the ear canal before surgery so that the tip of the tube microphone was less than 3 mm from the center of the eardrum. The ear canal was then plugged with a soft foam eartip (Etymotic Research). Once the facial recess was opened by the surgeon (Fig 2), the LDI system was positioned in such a way that the stapes (and, if possible, its footplate) was visible on the LDI built-in camera. The laser beam was aimed through the posterior tympanotomy onto the stapes, and the angle between the beam and the footplate was estimated. The multisine tone was generated and calibrated, and scanning measurements of the stapes vibration amplitude were performed as a baseline. The buttress (which is the bony bridge that remains after a posterior tympanotomy, connecting the lateral semicircular canal wall and the posterior auditory canal wall) was then carefully removed, and the PIL was sacrificed. However, the anatomic position of the incus was unchanged, and it was supported by medial fibers of the PIL that remained intact. The tympanic membrane also remained intact during the procedure. The operating microscope and the LDI could not be in position simultaneously, so that the LDI had to be

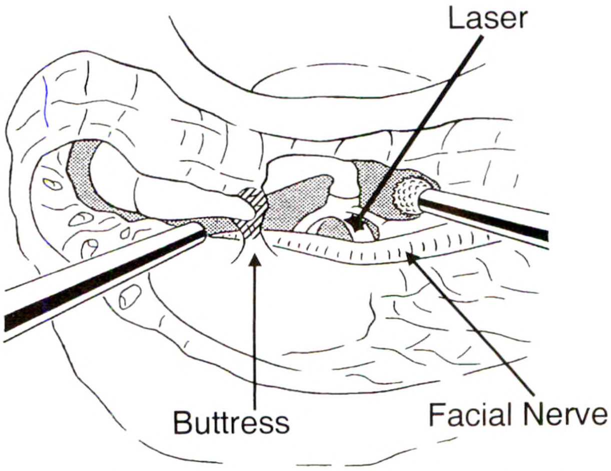


Fig 2. Schematic view of intraoperative measurement.

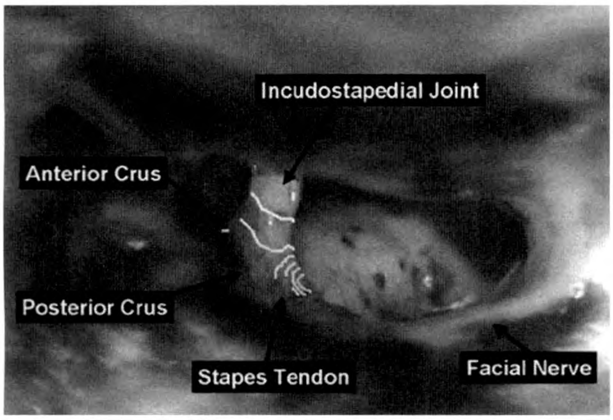


Fig 3. Stapes vibration seen through facial recess, graphically plotted as iso-amplitude lines at stimulation frequency of 500 Hz and 80 dB sound pressure level. Complex but mostly pistonlike movement is represented, with little rotational movement around long axis of stapes footplate and decreased movement at stapes tendon.

repositioned before the next measurement. The assessment of the stapes vibrations was then repeated, and the angle between the laser beam and the footplate was estimated again. For each measurement, a mean of 203 points (range, 69 to 295) was recorded on the stapes, incus, and promontory. The study included a mean of 20 (range, 7 to 31) reliable (signal coherence of 90% or higher) points on the stapes for each measurement.

RESULTS

Practicability. The experiment was performed within 10 to 15 minutes, including installing and aiming of the measuring system, calibration of the sound, and the scanning interferometry itself.

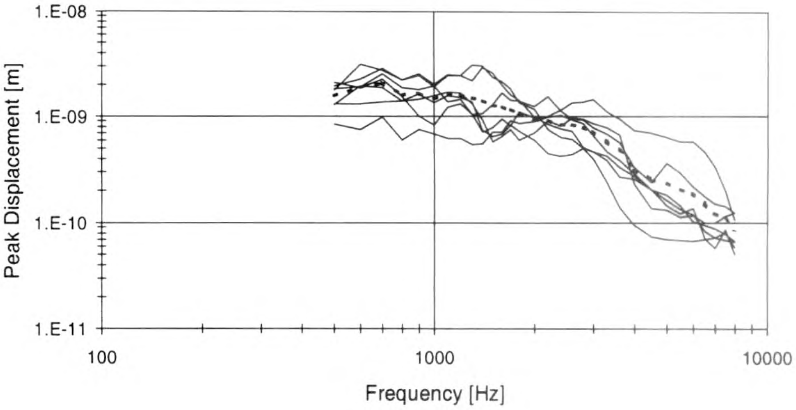
Figure 3 shows a representative measurement result of stapes vibration in one subject. Plotted are iso-amplitude lines at a stimulation frequency of 500 Hz and 80 dB SPL on the video image of the measured area. Each line represents an amplitude difference factor of 1.2. The highest vibration amplitude was found at the stapes head (1.9 nm), and the lowest was found at the stapes tendon (0.6 nm). Three-dimensional animation was used to qualitatively assess the vibration mode that showed complex but mostly pistonlike movement and no bending. At this frequency, the amplitude difference along the stapes only led to modest rocking.

Unlike in TB preparations, in live surgery the facial nerve is intact and limits the view of the stapes footplate. Therefore, in only 2 patients was the stapes footplate accessible to the laser. In the other 5 cases, the vibrations of the stapes superstructure were measured. The angle between the laser beam and an imaginary axis perpendicular to the stapes footplate was estimated to be 30° to 40° in all experiments, but did not differ by more than 10° in the same patient after repositioning of the LDI system.

Baseline Stapes Movement. Figure 4 shows the baseline displacement amplitudes of the stapes center in 7 cases. No angle correction was performed for the results. The peak displacement was approximately 2 nm in the frequencies below 1,000 Hz. The range was a factor of 3 to 10 (10 to 20 dB) over all frequencies. The repeatability within two readings of the same patient varied by about a factor of 1.12 (1 dB) when the LDI system was repositioned. By 3-dimensional animation of the data, the vibration mode of the stapes was found to be mostly pistonlike at lower frequencies up to about 1 kHz. At higher frequencies, a superimposed rocking motion became more dominant.

Posterior Incudal Ligament. In Fig 5, the mean stapes displacement amplitudes are plotted for baseline and a detached PIL. Although a minor increase in vibration amplitudes at lower frequencies and a slight decrease in vibration amplitudes at higher frequencies was found, these observations did not reach statistical significance according to the Mann-Whitney test. The maximum difference of the mean was a factor of 1.4 (2.9 dB) at 6,000 Hz. A difference factor of 0.6 to 1.8 (±5 dB) was not exceeded by any subject. Figure 5 also includes recently published measurements of 22 TB preparations performed with

Fig 4. Baseline displacement amplitude of stapes center (individual subjects and mean; n = 7).



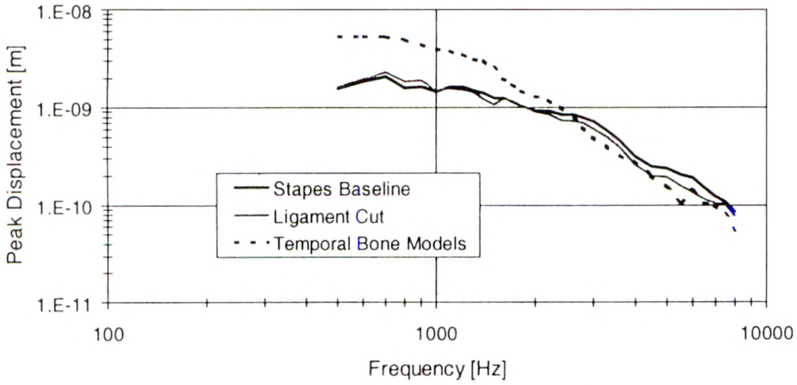


Fig 5. Mean stapes displacement amplitudes at baseline and after detaching posterior incudal ligament, and amplitudes of 22 temporal bone[7] preparations.

a similar technique through the facial recess.[7] The results differed at frequencies lower than 2,000 Hz and higher than 4,500 Hz by a maximum factor of 3 (10 dB).

DISCUSSION

Practicability. Our measurement system was easily applicable for intraoperative middle ear assessment, with only minimal prolongation (<15 minutes) of the surgery. The heavy sensor head and the scanning unit (12 kg) were suspended from a balanced operating microscope stand and were therefore comfortable for positioning. The laser light was sufficiently reflected from the object without any targets. Adequate signal-to-noise ratios and satisfying coherence of the signal were obtained.

Baseline Stapes Movement. In Fig 5, intraoperative stapes displacement measurements are compared to the results of 22 TB preparations. Although TB preparations are believed to exactly mimic the function in live humans, the postmortem effects caused by inner ear pressure change, tissue edema, temperature change, humidity, etc, cannot be ruled out. Guinan and Peake[3] concluded that stapes modes of vibration differ significantly in cadaver TBs and anesthetized cats and hypothesized that this difference is due to postmortem changes. Goode et al[8] found no difference in umbo vibration of live humans and TB preparations. With our method, it was for the first time possible to quantitatively assess in vivo the displacement amplitudes of the human stapes, which define the input into the inner ear, and to compare them to the results of cadaver TB experiments.

The results of intraoperative and TB experiments differed by less than 10 dB at low frequencies, and by less than 5 dB at high frequencies. Because the middle ear was unphysiologically opened to ambient air in the present study, the stapes vibrations are expected to be biased. On increasing the middle ear volume, Gyo et al[9] found improved sound transmission at lower frequencies (lower than 1 kHz) and decreased sound transmission at middle frequencies (1.5 to 4 kHz). According to these experiments, our results may be overestimated at lower frequencies and underestimated at higher frequencies by some decibels because of the lowered stiffening effect of the middle ear cavity. This effect adds to the difference between live human subject and TB experiments. On the other hand, the results from both the TB preparations and live humans are not angle-corrected. The measurement angle is almost perpendicular to the stapes footplate in the TB preparation because of the sacrificed facial nerve and the shortened posterior canal wall. This leads to a more accurate assessment of the stapes movement and higher displacement results. Further, the age groups of the studies do not match. The mean age was 74 years in the TB study and 31 years in this study. Although no statistically significant correlation of umbo vibration amplitude and age was found, young subjects tended to have a stiffer frequency response in a study by Goode et al.[8] This, again, may have led to a smaller displacement result in the present study. The differences, however, are in relation to the range of interindividual differences, which was approximately 10 to 20 dB in this study (Fig 4). We conclude that the TB is an adequate model for studying middle ear mechanics.

The stapes vibration was mainly pistonlike at lower frequencies and complex at frequencies above 1 kHz. This observation is in accordance with historic[4] and recent[5] studies in TBs and a recent study in anesthetized cats.[6] On the other hand, purely pistonlike[3] and purely rotational[1] forms of stapes movement were found over all frequencies by earlier authors. These measurements were performed with methods that did not allow the high level of accuracy afforded by modern LDI. Therefore, this discrepancy can be explained by the measurement sensitivity, especially at higher frequencies. Also, LDI measurements[2] have led to the conclusion that the stapes moves in a purely pistonlike way. In that study, the measured variability of different points on the stapes footplate was found to be up to 2 dB in amplitude and 20° in phase. This variability was described as due to the inaccuracy of the method and was less than in the present study. However, this variability could also represent significant rocking.

In accordance with experiments on stapes vibration in the TB,[5] no bending of the stapes was found at any frequency — a finding indicating rigid body behavior.

Posterior Incudal Ligament. The LDI measuring direction was not at right angles to the stapes footplate. If the major movement is assumed to be perpendicular to the stapes footplate, a systematic underestimation of the displacement amplitude in the range of 1 to 3 dB occurs. The measurement angle within each experiment, however, did not differ by more than 10°. The bias for assessment of the acoustic function of the PIL is therefore less than 1 dB and can be ignored. The stapes reflex was not induced during the experiments, because of the severe hearing loss of the subjects, and thus did not influence the trial.

The mean displacement amplitude of the stapes was not influenced by more than 2.9 dB by cutting of the PIL. All else being equal, this 2.9 dB represents a statistically nonsignificant ($p > .05$) change of the middle ear transfer function. The results are in accordance with the qualitative findings of von Békésy,[1] who did not find differences in middle ear transfer caused by cutting the PIL. This outcome implies that the PIL can be sacrificed during ear surgery without the risk of impairing the postoperative hearing result. However, this experiment does not take into consideration any of the postoperative changes (eg, scarring of the remaining PIL fibers) that may have an important impact on postoperative hearing.

Possible future applications of our intraoperative scanning LDI system are 1) assessment of stapes mobility in chronic ear surgery as a selection criterion for the best technique of hearing reconstruction, 2) quantitative detection of ossicular fixation in patients suffering from conductive hearing loss to assist in improving hearing results after surgery, 3) intraoperative quality control of ossiculoplasties and active middle ear implants, and 4) verification and improvement of mathematical middle ear models.

CONCLUSIONS

The described measurement system is easily applicable for intraoperative measurements and allowed for the first time quantitative assessment of the movement of the acoustically stimulated stapes with high accuracy during surgery. There are no major differences in terms of stapes vibration in live human subjects and cadaver TB preparations. Sacrificing the PIL has no immediate statistically significant effect on the middle ear transfer function.

ACKNOWLEDGMENT — The authors thank Ulrich Helfenstein, Department of Biostatistics, ISPM, University of Zurich, for his assistance in statistical analysis of the study results.

REFERENCES

1. von Békésy G. The action of the middle ear. In: Wever EG, ed. Experiments in hearing. New York, NY: McGraw-Hill, 1960:95-126.

2. Vlaming M, Feenstra L. Studies on the mechanics of the normal human middle ear. Clin Otolaryngol 1986;11:353-65.

3. Guinan J, Peake W. Middle-ear characteristics of anesthetized cats. J Acoust Soc Am 1966;34:1514-23.

4. Kirikae I. The structure and function of the middle ear. Tokyo, Japan: University of Tokyo Press, 1960.

5. Heiland K, Goode R, Asai M, Huber A. A human temporal bone study of stapes footplate movement. Am J Otol 1999; 20:81-6.

6. Decraemer W, Khanna S, Funnell W. Measurement and modeling of the three-dimensional vibration of the stapes in cat [Abstract]. Symposium on Recent Developments in Auditory Mechanics, Sendai, Japan, 1999.

7. Asai M, Huber A, Goode R. Analysis of the best site on the stapes footplate for ossicular chain reconstruction. Acta Otolaryngol (Stockh) 1999;119:356-61.

8. Goode R, Ball G, Nishihara S. Measurement of umbo vibration in human subjects — method and possible clinical applications. Am J Otol 1993;14:247-51.

9. Gyo K, Goode R, Miller C. Effect of middle ear modification on umbo vibration. Arch Otolaryngol Head Neck Surg 1986;112:1262-8.

Ann Otol Rhinol Laryngol 110:2001

MALIGNANT CAROTID BODY TUMORS: REPORT OF THREE CASES

LUIS PACHECO-OJEDA, MD
QUITO, ECUADOR

The aim of the current report was to describe 3 cases of malignant carotid body tumor (CBT) and to review the literature. My clinical records of 87 CBTs in 81 patients (6 bilateral) were reviewed, 79 of which were operated on. Three malignant cases were found. The first was in a 40-year-old man who presented with pulmonary metastases 6 years after resection of a CBT. He was treated with chemotherapy and interferon, but died with disseminated disease 2 years later. The second case was in a 56-year-old woman who had a 5-cm, fixed, hard mass in the upper aspect of the neck and a paralysis of the left vocal cord. This lesion was completely resected, and a shunt and reconstruction with a saphenous vein graft were performed. Pathology revealed a malignant chemodectoma with invasion to 2 of the 5 lymph nodes removed. Radiotherapy (50 Gy) was given after the operation. She is well and free of disease 68 months after the resection. The third case was in a 61-year-old woman who presented with an 8-cm nontender, hard, immobile mass in the left upper neck that displaced the left wall of the oropharynx toward the midline. A carotid arteriogram showed a CBT. On computed tomography, the tumor extended to the infratemporal fossa with no bone involvement. The lesion was embolized with a 40% reduction in vascularity. At surgical exploration, the tumor involved the sternocleidomastoid muscle and the lymph nodes at levels II and III, and the internal carotid artery could not be dissected free at the skull base, so only a partial resection was performed. This patient was lost to follow-up. These 3 cases are in agreement with the literature. Locoregional control is usually obtained with complete primary tumor resection and lymphadenectomy and eventual radiotherapy. Surgery with radiotherapy seems to be effective for isolated metastases. Current multidisciplinary treatments have been unsuccessful in controlling disseminated disease.

KEY WORDS — cervical chemodectoma, malignant carotid body tumor.

Chemodectomas are unusual neoplasms consisting of chemoreceptor cells occurring mainly at the carotid bifurcation. Since the first description by van Haller in 1743 and that by Marchand in 1891, approximately 1,200 cases of carotid body tumors (CBTs) have been reported. These lesions are more frequently found at high altitudes,[1] as in the Andean region of Ecuador, in South America.[2] The vast majority of these tumors are benign, but there is some disagreement about their metastatic potential (2% to 25%).[3] Thirteen metastatic CBTs were reviewed by Say et al[4] in 1973, and in 1985, Zbaren and Lehman[5] reviewed 106 cases with regional (57 cases) and distant (49 cases) metastases. Only 43 of them had detailed histologic documentation, and the most common site of metastasis was the lung (17 cases). Over a period of 18 years (1981 to 1998), my department has diagnosed 87 CBTs in 81 patients (6 bilateral). Seventy-nine tumors were operated on, all by the author. Three patients with malignant CBTs are described in this report.

CASE REPORTS

Case 1. A 40-year-old, apparently healthy man was admitted in the Social Security Hospital in Quito, Ecuador, in April 1983 with an asymptomatic, 6-cm, slow-growing lump in the left side of the neck. A carotid arteriogram showed a typical image of CBT. A resection under general anesthesia was performed without any operative incident. The postoperative course was uneventful. Macroscopic examination revealed a 6 × 5.5 × 3-cm lobulated, congested tumor surrounded by a thin connective membrane. Next to the tumor, 5 small lymph nodes were recognized. Microscopically, the tumor consisted of blood vessels lined with normal epithelium and surrounded peripherally by a solid proliferation of cuboidal and round cells. The diagnosis was carotid body chemodectoma and lymphatic hyperplasia.

In August 1991, this patient was admitted to another hospital for mild dyspnea and cyanosis. A chest x-ray revealed multiple small bilateral nodules (Fig 1). At bronchoscopy, congestive, hyperemic mucosae were visualized in both basal segments. The bron-

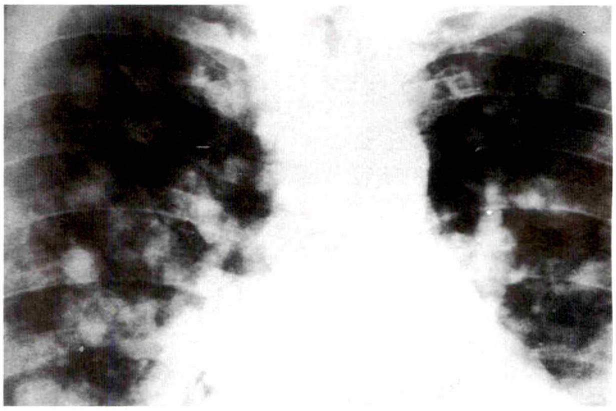


Fig 1. (Case 1) Chest x-ray shows multiple metastatic lesions.

From the Otorhinolaryngology and Head and Neck Surgery Service, Social Security Hospital, Quito, Ecuador.

CORRESPONDENCE — Luis Pacheco-Ojeda, MD, Grecia 175 y Mariana de Jesús, Quito, Ecuador.

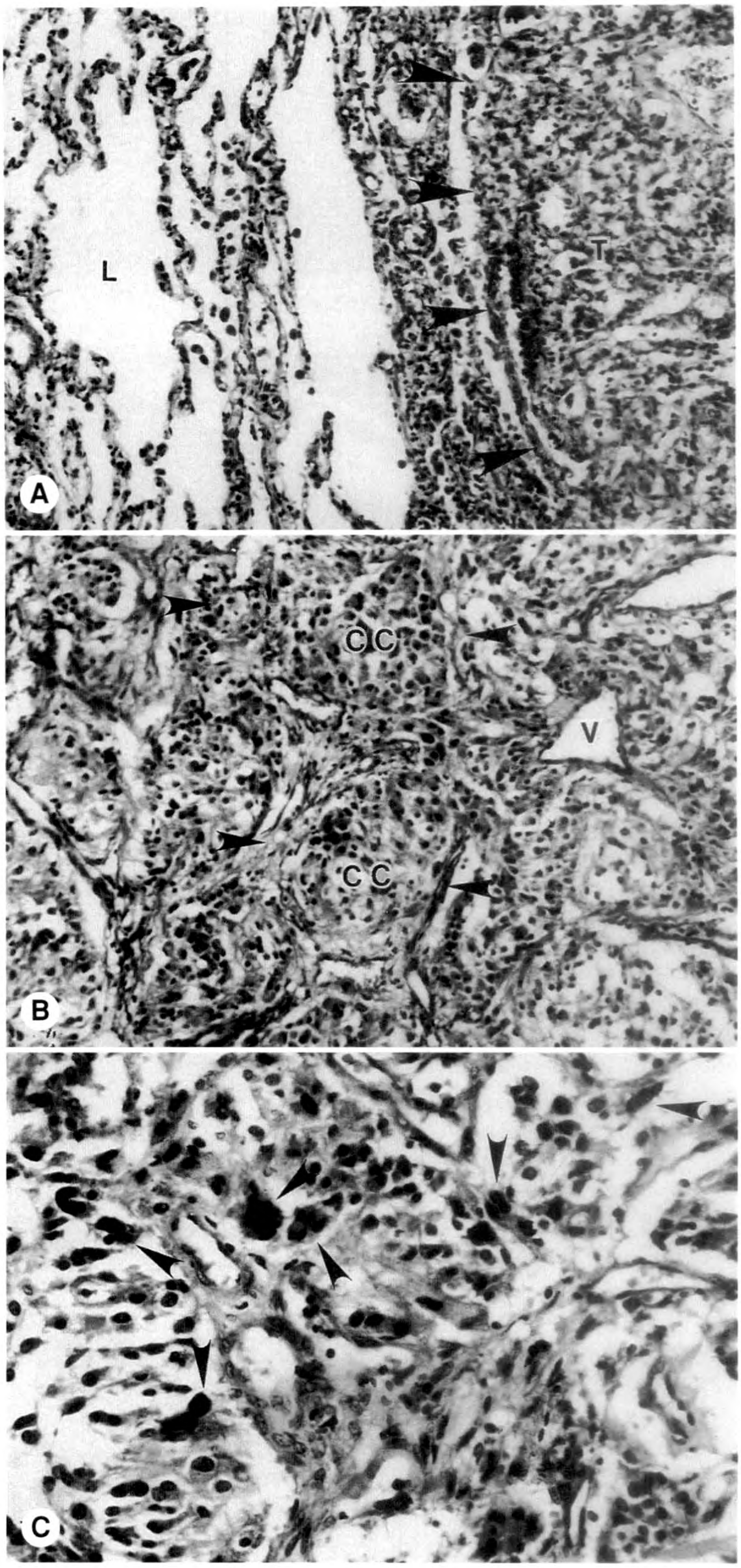


Fig 2. (Case 1) A) Pulmonary tissue (L) with atelectasis next to tumor capsule (arrowheads); metastatic tumor (T; original ×10). B) Tumor details: characteristic cluster cells (CC and arrowheads) and vascular canals (V; original ×40). C) Cellular details: atypical mitosis and atypical chromatic nuclei (arrowheads; original ×100).

chial biopsies and cytologic smears were negative for malignancy. Open pulmonary biopsies performed through a left thoracotomy on September 4, 1991, showed a 6 × 1.5-cm cuboid fragment covered by pleura and containing several hemorrhagic nodules, the biggest of which was 0.8 cm. Microscopically, the pulmonary tissue showed some subpleural nodules containing well-defined infiltrated foci of regu-

REVIEW OF MALIGNANT CAROTID BODY TUMORS REPORTED FROM 1986 TO 1999 (43 CASES)

			Treatment			
Disease Extent	*No. of Patients*		*Local*	*Regional*	*Metastases*	*Outcome*
Locally malignant	1		S + RT			NED at 3 y[6]
Locally malignant plus lymph nodes	4		S + RT	S + RT		NED at 5 y*
			S + RT	S + RT		DWD at 6 y[7]
			S + RT	S (1 RND) + RT		NED at 21 y[8]
			S (partial)	S		LFU*
Locally malignant plus metastases	1		NA		NA	NA[9]
Lymph nodes only	22	9		S (1 RND)		NED[7,10-15]
		11		S + RT		3 NED, 7 NA, 1 LR[16-19]
		2		NA		NA[20]
Lymph nodes plus metastases	3			S (1 RND)	S + RT	NED at 32 y[7]
				S	RT + CT	DWD at 4 y[21]
				S	None	DWD at 6 mo[14]
Metastases	12	2			S + RT	NA[22]
		1			RT	DWD[19]
		3			CT	DWD at 4 mo, 2 y, 8 y[23,24]*
		1			CT + RT	DWD at 4 y[23]
		1			None	DWD[25]
		4			NA	1 DWD at 2 y, 3 NA[26-29]

S — surgery, RT — radiotherapy, NED — no evidence of disease, DWD — died with disease, RND — radical neck dissection, LFU — lost to follow-up, NA — data not available, LR — local recurrence, CT — chemotherapy.

*Current report.

lar round-shaped cells. It was fibrous and well vascularized (Fig 2). The final diagnosis was pulmonary metastases of chemodectoma.

After an otherwise negative metastatic workup (except for the lungs), 2 courses of chemotherapy were given: cyclophosphamide 500 mg/m^2, doxorubicin 50 mg/m^2, and cis-platinum 50 mg/m^2. As no important regression occurred in the pulmonary nodules, 2b recombinant interferon 5,000,000 U was given 3 times a week for 3 months. This treatment was stopped because of progression of disease. The patient died 2 years after the discovery of the pulmonary metastases.

Case 2. A 56-year-old woman presented with dysphonia and an asymptomatic left neck lump that she had noted: a 5-cm, fixed, hard mass in the upper aspect of the neck. On indirect laryngoscopy, a paralysis of the left vocal cord was seen. A Doppler ultrasound study revealed minimal flow through the left carotid vessels, whereas the right carotid vessels had normal flow. Magnetic resonance imaging showed a characteristic vascular mass expanding and compressing the carotid bifurcation. A fine needle aspiration biopsy revealed few epithelial cells in a hemorrhagic environment.

A surgical resection was attempted, but only a partial resection and a limited lymphadenectomy was performed. Pathology revealed a malignant chemodectoma with invasion to 2 of the 5 lymph nodes removed. Two months later, the lesion was completely resected. The vagus nerve and the internal jugular vein were included in the resection. The hypoglossal and accessory nerves were preserved. A shunt and reconstruction with a saphenous vein graft were performed during the surgical procedure. The patient required a tracheostomy for 48 hours. Radiotherapy (50 Gy) was given after the operation. She is well and free of disease, 68 months after the resection.

Case 3. A 61-year-old woman presented with a 2-year history of dysphonia, asthenia, and weight loss. Mild dyspnea had appeared a few weeks previously. A tracheotomy had been performed upon arrival at the hospital. Examination revealed an 8-cm, nontender, hard, immobile mass in the upper half of the left side of the neck, displacing the left wall of the oropharynx toward the midline. A carotid arteriogram demonstrated a CBT. An ultrasound scan and a computed tomography (CT) scan revealed an enormous mass that displaced the pharynx and larynx to the right side. The tumor extended to the infratemporal fossa with no bone involvement. The lesion was embolized with a 40% reduction in vascularity. A surgical exploration was performed, but the internal carotid artery could not be dissected free at the skull base. The tumor involved the sternocleidomastoid muscle and the lymph nodes at levels II and III. It displaced but did not involve the larynx and the pharynx. No further attempt at resection was made in this patient, whose general condition was poor. She was sent for radiotherapy in the oncology department and was lost to follow-up.

DISCUSSION

Since the review by Zbaren and Lehman[5] in 1985, 55 additional cases of malignant CBT have been reported. Through research in Medline, I reviewed 50 cases reported in the world literature from 1986 to 1999.[6-30] Hence, the frequency of malignant CBT would be around 12%. This figure may be too high, as an unusual malignant case is more readily reported than a short series of benign cases. Data on the extent of disease, treatment, and outcome of 43 cases are summarized in the Table.[6-29] Most cases with local and regional malignancy were operated on and, consequently, did have histologic confirmation. Four cases reported as locally malignant, with or without regional disease, were treated with surgery and radiotherapy; only 1 patient died with disease. Twenty-two (51%) patients had isolated lymph node involvement, most of whom underwent a limited lymphadenectomy. In half of them, postoperative radiotherapy was delivered. No regional failure was reported. Distant metastases were reported in 16 patients (37%), all but 1 of whom are alive. Several multidisciplinary treatments were unsuccessful in controlling this disseminated disease.

There has been considerable debate in defining malignancy in CBT. Macroscopic (local invasion by continuity or contiguity) and microscopic criteria have been used by pathologists. Lack et al[31] described the following histologic characteristics of malignancy: central necrosis of the cell clusters, invasion of the vascular spaces, and mitosis. The tumor of my second patient had these characteristics. Other authors, however, have considered the presence of metastases, as in my first patient, as the only acceptable proof of malignancy.[20] Pantanowitz and Sareli[32] considered malignancy to be obvious in a case of multiple locally invasive chemodectomas of the carotid body and of the heart, which precluded a complete resection in both sites. The lesion in my third patient could be considered locally invasive under these criteria. Invasion to the vagus nerve, with subsequent vocal cord paralysis, is clinically suspicious of malignancy. Two of my patients and 1 in the series of Williams et al[7] presented initially with vocal cord paralysis. All of my 78 patients with benign lesions had normal vocal cord function.

The frequency of metastases to the regional lymph nodes is unknown. My second and third patients, the patient reported by Zbaren and Lehman,[5] and 25 patients of the current review had invasion to the adjacent lymph nodes. Neck dissection should be considered in such cases. Postoperative radiotherapy was delivered to half of the patients of the current review. The most common sites of distant metastases are the bones and the lungs.[5] The time interval between the resection of the primary tumor and the appearance of metastases was usually long and varied between 20 months and 20 years.[4,5,27] The time to metastasis was 6 years in my case 1. In reports with extended follow-up, metastasis occurred in as many as 30% of patients.[33,34] For this reason, I expect to have additional cases with metastatic disease with a longer follow-up.

The treatment of metastatic lesions has not been well defined, probably because of the rarity of their presentation. As the growth is usually slow (1 doubling every 2,000 days),[4] therapeutic abstention is a possibility. Surgery can be effective therapy in isolated metastases. As radiotherapy has been reported to be an effective method of achieving local control of CBT in a high proportion of patients,[35,36] it seems reasonable to use it in unresectable disease or bone metastases.[10,14,36]

Among the 17 cases of pulmonary metastases reviewed by Zbaren and Lehman,[5] only 1[37] was treated with a course of chemotherapy (actinomycin, vincristine, and cytoxan) without any change in the radiologic aspect of the pulmonary lesions. In a report of 6 metastatic chemodectomas (2 carotid body primaries) treated at Memorial Sloan Kettering Cancer Center,[4] there was no response to 8 different chemotherapeutic regimens. Radiotherapy was, on the contrary, very effective for palliation, particularly for bone metastases. A 9-year-old boy with pulmonary metastases and another patient with both nodal and hepatic metastases were treated with vincristine, cyclophosphamide, and doxorubicin (the former) and with carboplatin, cyclophosphamide, and doxorubicin (the latter), and no response was observed.[19,24] In a series of 13 paragangliomas in different sites of the body, there was a 46% partial remission with regimens that included cyclophosphamide, doxorubicin, and dacarbazine.[38] My first patient did not present any objective response to 2 courses of chemotherapy and interferon. This outcome is in agreement with the reported lack of chemosensitivity of this type of tumor.

Additionally, because in more than 50% of malignant cases there is regional disease, I am currently performing a limited lymphadenectomy of the neighboring superficial nodes — a maneuver that, in addition, allows a better exposure of the tumor.

REFERENCES

1. Saldana MJ, Salem LE, Travezan R. High altitude hypoxia and chemodectomas. Hum Pathol 1974;4:251-63.

2. Pacheco-Ojeda L, Durango E, Rodriguez C, Vivar N. Carotid body tumor at high altitudes: Quito, Ecuador, 1987. World J Surg 1988;12:856-60.

3. Merino MJ, Livolsi VA. Malignant carotid body tumors: report of two cases and review of the literature. Cancer 1981; 47:1403-14.

4. Say CC, Hori J, Spratt J. Chemodectoma with distant metastases: case report and review of the literature. Ann Surg 1973;39:333-41.

5. Zbaren P, Lehman W. Carotid body paraganglioma with metastasis. Laryngoscope 1985;95:450-4.

6. Rabl H, Friehs I, Guschi S, Pascher O, Koch G. Diagnosis and treatment of carotid body tumors. Thorac Cardiovasc Surg 1993;41:340-3.

7. Williams MD, Phillips MJ, Nelson WR, Rainer G. Carotid body tumor. Arch Surg 1992;127:963-8.

8. Cooper RA, Slevin NJ, Johnson RJ, Evans G. An unusual case of carotid body tumor. Clin Oncol (R Coll Radiol) 1998; 10:62-4.

9. Crouzet G, Vasdev A, Lambrinidis M, et al. Spinal metastases of carotid paraganglioma. One case and review of the literature. J Neuroradiol 1989;16:172-8.

10. McPherson GAD, Halliday AW, Mansfield AO. Carotid body tumors and other cervical paragangliomas: diagnosis and management in 25 patients. Br J Surg 1989;76:33-6.

11. Torres-Patiño F, Gomez-Acosta F, Guzman-Patraca C, Mendoza-Parada J, Labastida-Almedaro S. Tumor de cuerpo carotideo. Analisis de 96 casos. Rev Invest Clin 1991;43:119-23.

12. Wang D, Barros D'Sa AA, Johnston CF, Buchanan KD. Oncogene expression of carotid body tumors. Cancer 1996;77: 2581-7.

13. Barnes L, Taylor SR. Carotid body paragangliomas. A clinicopathologic and DNA analysis of 13 tumors. Arch Otolaryngol Head Neck Surg 1990;116:447-53.

14. Netterville JL, Reilly KM, Robertson D, Reiber ME, Armstrong WB, Childs P. Carotid body tumors: a review of 30 patients with 46 tumors. Laryngoscope 1995;105:115-26.

15. Dickinson PH, Griffin SM, Guy AJ, McNeill IF. Carotid body tumor: 30 year experience. Br J Surg 1986;73:14-6.

16. Rodriguez-Cuevas S, Lopez-Garza J, Labastida-Almendaro S. Carotid body tumors in inhabitants of altitudes higher than 2000 meters above sea level. Head Neck 1998;20:374-8.

17. Fruhwirth J, Koch G, Hauser H, Gutschi S, Beham A, Kainz J. Paragangliomas of the carotid bifurcation: oncological aspects of vascular surgery. Eur J Surg Oncol 1996;22:88-92.

18. Dinges S, Budach V, Stuschke M, Schmidt U, Budach W, Sack H. Malignant paragangliomas. The results of radiotherapy in 6 patients. Strahlenther Onkol 1993;169:114-20.

19. Defraigne JO, Sakalihassan N, Antoine P, Thiry A, Limet R. Carotid chemodectomas. Experience with nine cases with reference to preoperative embolization and malignancy. Acta Chir Belg 1997;97:220-8.

20. Ghilardi G, Bortolani EM, Pizzocari P, Vandone PL, De Monti M. Paraganglioma of the neck. Analysis of 32 operated cases. Minerva Chir 1991;46:1109-17.

21. Defraigne JO, Limet R. Lymphatic, hepatic and osseous metastasis of a carotid chemodectoma. A propos of a case. J Chir (Paris) 1997;134:336-9.

22. Kawai A, Healey JH, Wilson SC, Huvos AG, Yeh SD. Carotid body paraganglioma metastatic to bone: report of two cases. Skeletal Radiol 1998;27:103-7.

23. Massey V, Walkner K. Treatment of metastatic chemodectoma. Cancer 1992;69:790-2.

24. Hajnzic TF, Kruslin B. Carotid body paraganglioma in a nine-year old boy with extensive pulmonary metastases. Med Pediatr Oncol 1999;32:399-400.

25. Guerrier B, Makeieff M, Louche C, Mouketou JB, Crampette L. Paragangliomes cervicaux. Resultats à propos d'une serie de 33 patients. J Chir (Paris) 1995;132:287-94.

26. Nora JD, Hallett JW, O'Brien PC, Naessens JM, Cherry KJ, Pairolero PC. Surgical resection of carotid body tumors: long-term survival, recurrence and metastasis. Mayo Clin Proc 1988;63:348-52.

27. North CA, Zinreich ES, Christensen WN, North RB. Multiple spinal metastases from paraganglioma. Cancer 1990;66: 2224-8.

28. Mitchell RO, Richardson JD, Lambert GE. Characteristic, surgical management, and outcome in 17 carotid body tumors. Am Surg 1996;62:1034-7.

29. Westerband A, Hunter GC, Cintora I, et al. Current trends in the detection and management of carotid body tumors. J Vasc Surg 1998;28:84-92.

30. Gaylis H, Davidge-Pitts K, Pantanowitz D. Carotid body tumors. A review of 52 cases. S Afr Med J 1987;72:493-6.

31. Lack EE, Cubilla AL, Woodruff JM. Paragangliomas of the head and neck region. A pathological study of tumors from 71 patients. Hum Pathol 1979;10:191-218.

32. Pantanowitz D, Sareli P. Multiple malignant paragangliomas. A case report. S Afr Med J 1989;76:441-3.

33. Gaylis H, Mieny CJ. The incidence of malignancy in carotid body tumors. Br J Surg 1977;64:885-9.

34. Martin CE, Rosenfeld L, McSwain B. Carotid body tumors: a 16-year follow-up of seven malignant cases. South Med J 1973;66:1236-46.

35. Konefal JB, Pilepich MV, Spector GJ, Perez CA. Radiation therapy in the treatment of chemodectomas. Laryngoscope 1987;97:1331-5.

36. Valdagni R, Anichetti M. Radiation therapy of carotid body tumors. Am J Clin Oncol 1990;13:45-8.

37. Tu H, Bottomley RH. Malignant chemodectoma presenting as miliary pulmonary infiltrate. Cancer 1974;33:244-9.

38. Patel SR, Winchester DJ, Benjamin RS. A 15-year experience with chemotherapy of patients with paraganglioma. Cancer 1995;76:1476-80.

Ann Otol Rhinol Laryngol 110:2001

FURTHER EXPERIENCE WITH TRACHEOJEJUNAL SHUNT SPEECH AFTER PHARYNGOLARYNGOESOPHAGECTOMY

MINORU KINISHI, MD

MUTSUO AMATSU, MD SHINYA TAHARA, MD

KOBE, JAPAN

Over the past 10 years, 16 patients have undergone the tracheojejunal shunt operation for voice reconstruction after undergoing pharyngolaryngoesophagectomy with free jejunum reconstruction for advanced hypopharyngeal cancer. For the purpose of voice reconstruction, a 2-cm inferiorly based tracheal flap is obtained from the membranous part of the trachea by removing 4 cartilaginous tracheal rings. After the establishment of digestive continuity with the jejunal graft, a side-to-side anastomosis is created by approximating the incised margin of the jejunal mucosa to that of the tracheal flap. The tracheal flap is tubed to construct the tracheojejunal shunt. In addition, the incised margin of the jejunal serosa is sutured to the lateral wall of the shunt to reinforce the approximation of the shunt to the jejunal graft. Thirteen of the 16 patients (81%) were initially capable of voice production. The length of time during which tracheojejunal speech has been used ranges from 18 to 122 months, with a mean of 55 months. During follow-up, 12 of the 13 patients (92%) have been able to swallow without aspiration.

KEY WORDS — advanced hypopharyngeal cancer, free jejunal autograft, pharyngolaryngoesophagectomy, tracheojejunal shunt, voice reconstruction.

INTRODUCTION

Among the variety of surgical reconstruction methods so far reported, the use of a revascularized free jejunal segment is one of the most reliable options currently available for reconstructing the discontinuity of the upper digestive tract after pharyngolaryngoesophagectomy. Although the restoration of swallowing function is, needless to say, mandatory, an immediate voice reconstruction procedure should be attempted and evaluated as well, to rescue patients from a devastating, voiceless life following surgery.

Very few attempts at such voice reconstruction have been recorded.[1,2] On the basis of the satisfactory results of postlaryngectomy voice reconstruction with the tracheoesophageal (TE) shunt procedure using the membranous part of the trachea,[3-5] we successfully performed a tracheojejunal (TJ) shunt operation in 3 patients.[6] The basic idea of the technique, like that of the TE procedure, is to divert pulmonary air through the TJ shunt into the transplanted jejunum, where vibration occurs and sound is produced. This paper reports 10 years' experience with the TJ shunt procedure in 16 patients and discusses the functions of speech and swallowing.

SUBJECTS AND METHODS

From 1988 through 1997, 16 patients (10 male, 6 female) with advanced hypopharyngeal cancer underwent the TJ shunt procedure at the time of pharyngolaryngoesophagectomy with a free jejunum reconstruction. All underwent ipsilateral radical and contralateral elective neck dissections. The characteristics of the patients thus treated are listed in Table 1. The patients' ages at surgery ranged from 40 to 73 years, with a mean of 61 years. One patient received preoperative radiotherapy of 40 Gy, and another received 50 Gy after the operation. Four underwent adjuvant chemotherapy, and 2 underwent neoadjuvant chemotherapy. The follow-up ranges from 11 to 125 months, with a mean of 54 months.

A previous article of ours contains a complete and illustrated description of the TJ shunt procedure for voice reconstruction.[6] The original technique has been modified and refined, resulting in a simple surgical procedure. A 2-cm inferiorly based tracheal flap is obtained from the membranous part of the trachea by removing 4 cartilaginous tracheal rings. After the establishment of digestive continuity with the jejunal graft, a short midline vertical incision of 8 mm is made, starting just below the superior margin of the tracheal flap. A vertical incision of free jejunum corresponding to that of the tracheal flap is carried out to enter the jejunal lumen (see Figure, A). A side-to-side anastomosis is created by approximating the incised margin of the jejunal mucosa to that of the tracheal flap through the incised slit of the tracheal flap with 8 stitches of absorbable thread (see Figure, B).

From the Departments of Otorhinolaryngology–Head and Neck Surgery (Kinishi, Amatsu) and Plastic and Reconstructive Surgery (Tahara), Kobe University School of Medicine, Kobe, Japan.

CORRESPONDENCE — Minoru Kinishi, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, Kobe University School of Medicine, 650-0017 Kusunoki-cho 7-5-1, Chuo-ku, Kobe, Japan.

TABLE 1. PATIENTS AND DISEASE DATA

Patient No.	*Age*	*Sex*	*Tumor Subsite*	*TNM Classification*	*Combined Treatment*	*Follow-up*
1	57	F	Posterior wall	T4N1M0	None	75 mo, DOAC
2	73	F	Posterior wall	T4N0M0	Preop 40 Gy + AC	125 mo, alive
3	63	M	Pyriform sinus	T3N0M0	Postop 50 Gy + AC	20 mo, DOAC
4	69	F	Pyriform sinus	T2N2cM0	AC	73 mo, DOAC
5	62	F	Posterior wall	T4N0M0	None	121 mo, alive
6	66	M	Pyriform sinus	T3N1M0	AC	28 mo, DOAC
7	53	M	Postcricoid	T3N0M0	None	66 mo, DOAC
8	61	M	Pyriform sinus	T3N0M0	None	69 mo, DOAC
9	71	M	Pyriform sinus	T3N3M0	NAC	11 mo, DOD
10	54	F	Pyriform sinus	T3N0M0	None	79 mo, alive
11	58	M	Postcricoid	T3N1M0	None	44 mo, DOD
12	40	F	Pyriform sinus	T3N3M0	NAC	25 mo, DOD
13	62	M	Pyriform sinus	T4N0M0	None	32 mo, DOAC
14	65	M	Pyriform sinus	T4N0M0	None	45 mo, alive
15	69	M	Pyriform sinus	T2N1M0	None	31 mo, alive
16	56	M	Postcricoid	T3N0M0	None	20 mo, alive

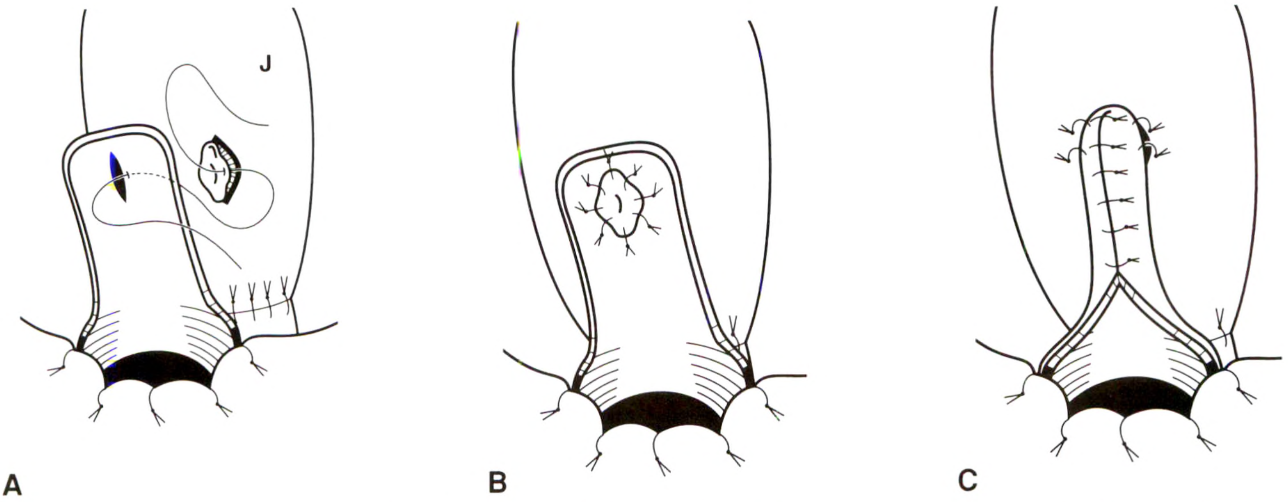
DOAC — died of another cause; AC — adjuvant chemotherapy; NAC — neoadjuvant chemotherapy; DOD — died of disease.

The lateral margins of the tracheal flap are approximated from above with absorbable thread to construct the TJ shunt. The incised margin of the jejunal serosa is also sutured to the lateral wall of the shunt to reinforce the approximation of the shunt to the jejunal graft (see Figure, C).

Speech intelligibility was psychoacoustically evaluated by the family of each patient and 1 of the authors, and rated on a scale of 1 to 5 on an equal-appearing–interval scale according to the evaluation method proposed by Hirose.[7] A total score of 8 to 10 was considered "excellent" speech, 5 to 7 was "moderate," and 2 to 4 was "poor" (Table 2).

RESULTS

Data on voice production, time to initial phonation, maximum phonation time (MPT), length of time during which TJ speech was used, intelligibility ratings[7] of TJ speech, and aspiration problems are given in Table 3. Thirteen of the 16 patients (81%) were initially capable of voice production. All of these pa-



Procedure. A) Two-centimeter inferiorly based tracheal flap is obtained from membranous part of trachea by removing 4 cartilaginous tracheal rings. After establishment of digestive continuity with jejunal graft (J), incisions of tracheal flap and transplanted jejunum are made. B) Side-to-side anastomosis is created by approximating incised margin of jejunal mucosa to that of tracheal flap through incised slit of tracheal flap. C) Lateral margins of tracheal flap are approximated to construct tracheojejunal shunt. Incised margin of jejunal serosa is sutured to lateral wall of shunt to reinforce approximation of shunt and jejunal graft.

TABLE 2. EVALUATION OF SPEECH BY HIROSE[7] METHOD

	Score	
Grade of Intelligibility	*By Family (A)*	*By Non-family (B)*
Understandable	5	5
Occasionally not understandable	4	4
Understandable when topic is limited	3	3
Occasionally understandable	2	2
Not understandable	1	1

Judgment (A + B): 8 to 10 — excellent; 5 to 7 — moderate; 2 to 4 — poor.

tients continued to use TJ speech until the end of the follow-up period or until they died. Two patients failed to acquire a voice because of stenosis of the jejunal orifice of the TJ shunt, and the remaining patient could not use TJ phonation because of poor pulmonary reserve that developed after the operation.

Phonation was initiated on the 33rd postoperative day, on average (range, 19 to 51 days). The MPT ranged from 6 to 16 seconds, with a mean of 12 seconds. The length of time during which TJ speech was used ranged from 18 to 122 months, with a mean of 55 months. As far as the intelligibility evaluation by the family of each patient and 1 of the authors is concerned, 3 patients had excellent speech clarity, 9 moderate, and 1 poor.

All patients could swallow solid foods and liquids without dysphagia or regurgitation. Of the 13 patients who had voice capability with the TJ shunt, 12 could swallow without aspiration during the course of follow-up. The remaining patient developed mild aspiration of liquids 4 months after revision surgery of the tracheostoma.

DISCUSSION

Although the prognosis of most patients with advanced hypopharyngeal cancer who undergo pharyngolaryngoesophagectomy with bilateral neck dissection remains bleak, their postoperative quality of life is improved with immediate 1-stage reconstruction of the upper digestive tract. To date, few attempts at voice reconstruction have been reported. Saito et al[1] reported a tracheogastric shunt method to be used at the time of gastric pull-up reconstruction after total pharyngolaryngoesophagectomy. Although patients can produce a substitute voice with this method, the requirement of unnecessary resection of the intact distal esophagus is a disadvantage. Ehrenberger et al[2] proposed a siphon-like tracheopharyngeal shunt using a free jejunum interposition. Although the diameter of the shunt offers low flow resistance, the use of transplanted jejunum to connect the trachea to the hypopharynx or jejunal segment entails the possibility that jejunal secretions may directly enter the trachea.

Vocal rehabilitation after laryngectomy with or without neck dissection has been established, and consists of esophageal speech, an artificial larynx, or an additional operative procedure to construct a shunt connecting the respiratory tract to the upper digestive tract. Over the past 4 decades, a variety of surgical voice reconstruction procedures have been reported and widely put into practice.[8-10] The TE shunt procedure, with or without a prosthesis, has become

TABLE 3. SPEECH AND ASPIRATION DATA

Patient No.	*Voice Production*	*Time to Initial Phonation (d)*	*MPT (s)*	*Length of Time With TJ Speech (mo)*	*Intelligibility of TJ Speech**	*Aspiration Problem*
1	Success	19	15	73	8 (4 + 4)	None
2	Success	51	13	122	8 (4 + 4)	None
3	Success	30	10	18	5 (3 + 2)	None
4	Failure					
5	Success	31	15	120	7 (4 + 3)	None
6	Success	38	12	26	5 (3 + 2)	None
7	Success	20	11	65	6 (3 + 3)	None
8	Success	21	11	67	5 (3 + 2)	None
9	Failure					
10	Success	45	15	77	8 (4 + 4)	None
11	Success	30	16	43	7 (4 + 3)	None
12	Success	50	10	23	6 (3 + 3)	None
13	Success	30	6	30	4 (2 + 2)	None
14	Failure					
15	Success	47	15	29	6 (3 + 3)	None
16	Success	23	10	19	6 (3 + 3)	Minimum

MPT — maximum phonation time, TJ — tracheojejunal shunt.

*Total score by Hirose[7] method (score given by family + score given by 1 of authors).

a well-established and reliable method of choice for speech rehabilitation after laryngectomy.[5,11]

In the present series, 13 of the 16 patients who underwent the TJ shunt procedure had voice capability, and the longest follow-up reached over 10 years. However, the MPT and speech intelligibility of TJ speakers were not satisfactory in comparison with those of TE speakers.[12] In TE speech, the innervated thyropharyngeus muscle of the remnant pharyngeal wall plays an important role in producing audible and intelligible voice.[13-16] On the other hand, the vibratory source in TJ speech is merely located at the stenotic portion produced by unmodulated natural peristalsis, because pharyngolaryngoesophagectomy requires resection of the thyropharyngeus muscle. The decisive difference between TJ and TE speech is that the jejunal autograft in TJ speech does not provide as good a vibratory source as does the thyropharyngeus muscle in TE speech. Although TJ speech does not possess the regulating mechanism provided by the thyropharyngeus muscle, the TJ shunt procedure does provide patients with useful speech.

Although aspiration is a problem to be solved in any surgical procedure for voice reconstruction, it was indeed minimized in this series. The intricate structure of the jejunal mucosa, which consists of circular folds and intestinal villi, covers the jejunal orifice at the bottom of the shunt, between the consecutive circular folds. The intraluminal pressure of the transplanted jejunum during natural peristalsis and swallowing of water boluses did not reach 10 mm Hg.[17] This was a lower value than the esophageal pressure of 30 mm Hg measured during swallow in laryngectomized patients.[18] In the only patient in whom mild aspiration developed in this series, incision of the anterior wall of the TJ shunt altered its course, so that the anterior covering of the shunt was lacking. It seems reasonable to conclude that the above-described characteristics — intricate anatomic structure, lower intraluminal pressure in the transplanted jejunum, and anterior covering of the shunt — are effective in precluding liquids and jejunal secretions from entering the shunt.

We have described our 10 years of experience with the TJ shunt procedure in patients who underwent pharyngolaryngoesophagectomy with a free jejunal graft for reconstruction. It is concluded that the TJ shunt procedure is successful in providing useful speech and in minimizing aspiration in these patients.

REFERENCES

1. Saito H, Sato F, Saito A, et al. Vocal rehabilitation by tracheogastric shunt method after pharyngolaryngoesophagectomy for malignancy. Arch Otorhinolaryngol 1984;240:35-41.

2. Ehrenberger K, Wicke W, Piza H, Roka R, Grasl M, Swoboda H. Jejunal grafts for reconstructing a phonatory neoglottis in laryngectomized patients. Arch Otorhinolaryngol 1985;242:217-23.

3. Amatsu M, Matsui T, Maki T, Kanagawa K. Vocal rehabilitation after total laryngectomy: a new one-stage surgical technique. J Otolaryngol Jpn 1977;80:779-85.

4. Amatsu M. A one stage surgical technique for postlaryngectomy voice rehabilitation. Laryngoscope 1980;90:1378-86.

5. Amatsu M, Makino K, Kinishi M, Tani M, Kokubu M. Primary tracheoesophageal shunt operation for postlaryngectomy speech with sphincter mechanism. Ann Otol Rhinol Laryngol 1986;95:373-6.

6. Kinishi M, Amatsu M, Tahara S, Makino K. Primary tracheojejunal shunt operation for voice restoration following pharyngolaryngoesophagectomy. Ann Otol Rhinol Laryngol 1991;100:435-8.

7. Hirose H. The evaluating method of speech. In: Japan Society for Head and Neck Cancer. General rules for clinical and pathological studies on head and neck cancer. 2nd ed. Tokyo, Japan: Kanehara, 1991:101.

8. Conley JJ, DeAmesti F, Pierce MK. A new surgical technique for the vocal rehabilitation of the laryngectomized patient. Ann Otol Rhinol Laryngol 1958;67:655-64.

9. Asai R. Asai's new voice production method: a substitution for human speech [Abstract]. Proceedings of 8th International Congress of Otorhinolaryngology, Tokyo, Japan, 1965:730.

10. Staffieri M, Serafini I. La riabilitazione chirurgica della voce er della respirazione dopo laringectomia totale. In: Fidenza M, ed. 29th National Congress of the Associazione Otologi Ospedalieri Italiana, Bologna, Italy, 1976:57-111.

11. Singer MI, Blom ED. An endoscopic technique for restoration of voice after laryngectomy. Ann Otol Rhinol Laryngol 1980;89:529-33.

12. Amatsu M, Kinishi M, Jamir JC. Evaluation of speech of laryngectomees after the Amatsu tracheoesophageal shunt operation. Laryngoscope 1984;94:696-701.

13. Yoshifuji M, Mohri M, Kinishi M, Amatsu M. A study of the neoglottis in tracheoesophageal phonation. J Jpn Bronchoesophagol Soc 1994;45:13-21.

14. Mohri M, Yoshifuji M, Kinishi M, Amatsu M. Neoglottic activity in tracheoesophageal phonation. Auris Nasus Larynx 1994;21:53-8.

15. Fujimoto T, Kinishi M, Mohri M, Amatsu M. Mechanism of neoglottic adjustment for voice variation in tracheoesophageal speech. J Otolaryngol Jpn 1994;97:1009-18.

16. Teraoka M, Kinishi M, Mohri M, Amatsu M. Neoglottic articulatory adjustment in tracheoesophageal speech. Larynx Jpn 1995;7:35-42.

17. Kerlin P, McCafferty GJ, Robinson DW, Theile D. Function of a free jejunal "conduit" graft in the cervical esophagus. Gastroenterology 1986;90:1956-63.

18. Amatsu M. Sphincter mechanism against aspiration in tracheoesophageal speaker. In: Amatsu M, ed. Surgical voice reconstruction following laryngectomy. Kobe, Japan: Kobe University School of Medicine, 1992:165-75.

Ann Otol Rhinol Laryngol 110:2001

PERFORMANCE CHARACTERISTICS OF FLUORESCENCE ENDOSCOPE IN DETECTION OF HEAD AND NECK CANCERS

BOONCHU KULAPADITHAROM, MD VIPA BOONKITTICHAROEN, PHD

BANGKOK, THAILAND

Laser-induced fluorescence endoscopy (LIFE) is a noninvasive method for detecting early cancers in hollow organs. A laser-induced fluorescence endoscope that was designed for lung imaging was investigated for its effectiveness in comparison to white light endoscopy (WLE) in localizing head and neck cancers. A total of 196 images from 98 sites in 56 patients were graded into 5 levels from normal to definitely abnormal. A cutoff level to differentiate normal from abnormal images was chosen from a receiver operating characteristic curve. On this basis, LIFE (sensitivity, 92.86%; specificity, 78.57%) was observed to be more effective and reliable than WLE (sensitivity, 67.86%; specificity, 70%) in locating neoplastic foci and precancerous lesions. Nevertheless, WLE was helpful in presenting anatomic details. The reliability of LIFE varied from site to site. It excelled in examination of the oropharynx (sensitivity, 100%; specificity, 96.20%), but was less effective for lesions of the nasopharynx (sensitivity, 66.70%; specificity, 75.00%). In standardizing the test, interobserver variation was assessed, and good agreement in image interpretation was confirmed by statistical analysis. In conclusion, LIFE was found to be an effective and reliable tool for detecting head and neck cancers.

KEY WORDS — head and neck cancer, laser-induced fluorescence endoscope, tumor imaging, white light endoscope.

INTRODUCTION

Head and neck tumorigenesis is best described by two important concepts: multistep carcinogenesis and field cancerization.[1,2] On this basis, dysplasia, carcinoma in situ, and invading cancer may arise either synchronously or metachronously at different sites in the upper aerodigestive tract, in which the entire mucosal surface is likely to be exposed to the same carcinogens.[1,2] A common consensus on the most effective means of controlling head and neck cancers, whenever possible, is to treat either premalignant lesions or malignancy in the early stage in order to reverse or suppress the carcinogenic process.[1,2] Detection of field cancers that present at different stages of malignancy remains a long-standing problem for most surgical oncologists. Ideally, there is a need for a sensitive and efficient detection device with the ability to search the upper aerodigestive tract extensively for sites of premalignant lesions or early malignant lesions that are visually undetectable by standard endoscopic investigation.

Laser-induced fluorescence (LIF) spectroscopy has gained widespread recognition for its potential in tissue diagnosis.[3-7] In an animal tumor that is induced by a chemical carcinogen, ie, the hamster cheek pouch model, LIF spectroscopy is able to reflect spectral changes corresponding to tissue progression from normal mucosa to dysplasia and neoplasia.[8] In clinics, LIF spectroscopy is capable of identifying malignant and premalignant lesions in various organs and regions, eg, head and neck,[7] esophagus,[6] lung,[4] colon,[3] and cervix.[5] Contact fluorometry[3,5,6] and fluorescence imaging[4,7] are the two standard techniques applied during endoscopic examination. As broad-field fluorescence imaging is easier to perform than contact fluorometry, it possesses more potential than small-field contact fluorometry for detecting premalignant or early malignant lesions or sites.

One of the most interesting systems for lung imaging is laser-induced fluorescence endoscopy (LIFE), which was developed by Palcic et al[9]; this system has been tested and shown to be superior to white light bronchoscopy in revealing dysplasia and carcinoma in situ in the lung.[4] We demonstrated the potential of LIFE in early detection of head and neck cancers.[7]

In this study, various performances of LIFE in the examination of the head and neck region were characterized and analyzed in order to acquire basic data for system standardization and for unbiased interpretation of LIF spectroscopic findings.

MATERIALS AND METHODS

Patients. In this prospective study, 56 patients in whom cancer(s) in the head and neck was suspected underwent LIFE examination. A total of 98 sites were investigated and submitted to biopsy for histopathologic diagnosis.

Endoscopic Procedure. Under local anesthesia, the

From the Department of Otolaryngology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

CORRESPONDENCE — Boonchu Kulapaditharom, MD, Dept of Otolaryngology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.

TABLE 1. RATIOS OF LIKELIHOOD OF DIFFERENT GRADES OF IMAGING FINDINGS TO SUGGEST PRESENCE OF MALIGNANCY

Imaging	*Grade*	*Finding*	*Likelihood Ratio*	*Interpretation*
WLE	5	Ulcerative growth, irregular mucosa	∞	Definitely abnormal
	4	Growth with or without color changes	2.49	Probably abnormal
	3	Raised lesion with or without color changes	0.83	Possibly abnormal
	2	Smooth mucosa with color changes	1.67	Probably abnormal
	1	Normal mucosa	0.46	Probably normal
LIFE	5	Focal dark area with clustered brown–rusty red dots	14.04	Definitely abnormal
	4	Focal dark area	4.07	Probably abnormal
	3	Diffuse dark area with clustered brown–rusty red dots	1.00	Possibly abnormal
	2	Diffuse dark area	0.36	Probably normal
	1	Green area with or without scattered red dots	0	Definitely normal

WLE — white light endoscopy, LIFE — laser-induced fluorescence endoscopy.

patients were first examined by white light fiberoptic bronchoscopy; an Olympus BF 20D bronchoscope was connected to a charge-coupled device (CCD) camera. The areas under investigation were the oral cavity, nasal cavity, nasopharynx, oropharynx, hypopharynx, and larynx. For comparison, the white light source was then switched to a helium-cadmium laser (442 nm). The fiberoptic endoscope was then attached to a LIFE camera unit that had 2 image-intensified CCD cameras incorporated with red (630 nm and longer) and green (480 to 520 nm) filters. Both the white light and fluorescence images were displayed on a color video monitor; these images were then selected and stored in the system computer. A biopsy was performed when any alteration in structure or color was recognized either by white light endoscopy (WLE) or LIFE.

Image Characterization. The white light images were graded into 5 categories ranging from normal mucosa to mucosal thickening to neoplasm (Table 1). Three major patterns of fluorescence images — a focal dark area with or without clusters of brown–rusty red dots, a diffuse dark area with or without clusters of brown–rusty red dots, and a green area with or without scattered brown–rusty red dots — were rated into 5 grades (Table 1). After obtaining more information on the biopsy results, we calculated likelihood ratios related to the likelihood of detection of diseases for each grade.[10] Finally, the endoscopic findings were defined in relation to histopathologic findings as true-positive or false-negative for dysplasia and neoplasia and as true-negative or false-positive for normal mucosa, granuloma, and inflammation.

Receiver Operating Characteristic Curve. By successively relaxing the cutoff level from high- to low-grade images (eg, grade 5, grades 5 plus 4, and so on), we obtained a different combination of true- and false-positive rates for each cutoff point. The data were plotted on a double probability paper to obtain a receiver operating characteristic (ROC) curve,[10,11] and the area under the curve was calculated by the method suggested by Hanley and McNeil.[12] A statistical analysis for the difference between the areas of the WLE and LIFE ROC curves was performed by choosing a 95% statistical power.[12,13]

Interobserver Variability. Six investigators — 2 medical staff and 4 residents — who were familiar with the concept of fluorescence imaging were familiarized with the nature of the fluorescence image. For simplicity, the fluorescence gradings (5 to 1, as shown in Table 1) were transformed into confidence percentages (100%, 75%, 50%, 25%, and 0%) reflecting the confidence levels that the image was abnormal. The cutoff point for negative and positive findings was selected at the 50% confidence level. Each observer was asked to rank independently a series of 26 fluorescence images as normal, inflammation, granuloma, dysplasia, or neoplasia. Without knowledge of the biopsy findings, the investigators were briefed case-by-case about the findings from clinical examinations and then were presented with pairs of white light and fluorescence images for assessment. Image readings by an expert were used as a reference for interobserver comparison. The degree of interobserver agreement on image reading was determined by the magnitude of κ, a ratio of actual to potential agreement.[10,11] A McNemar bias index was used to calculate and to reflect the magnitude of bias when two observers disagreed.[11] A value of 1 would suggest the maximum degree of disagreement.[11] Significant bias was tested by a McNemar χ^2 test.[11,13] Variations in image rating among observers were also evaluated by analysis of variance by rank using a Kruskal-Wallis test.[13]

RESULTS

Characteristics of White Light and Fluorescence Images. In 56 patients, 98 sites were investigated pro-

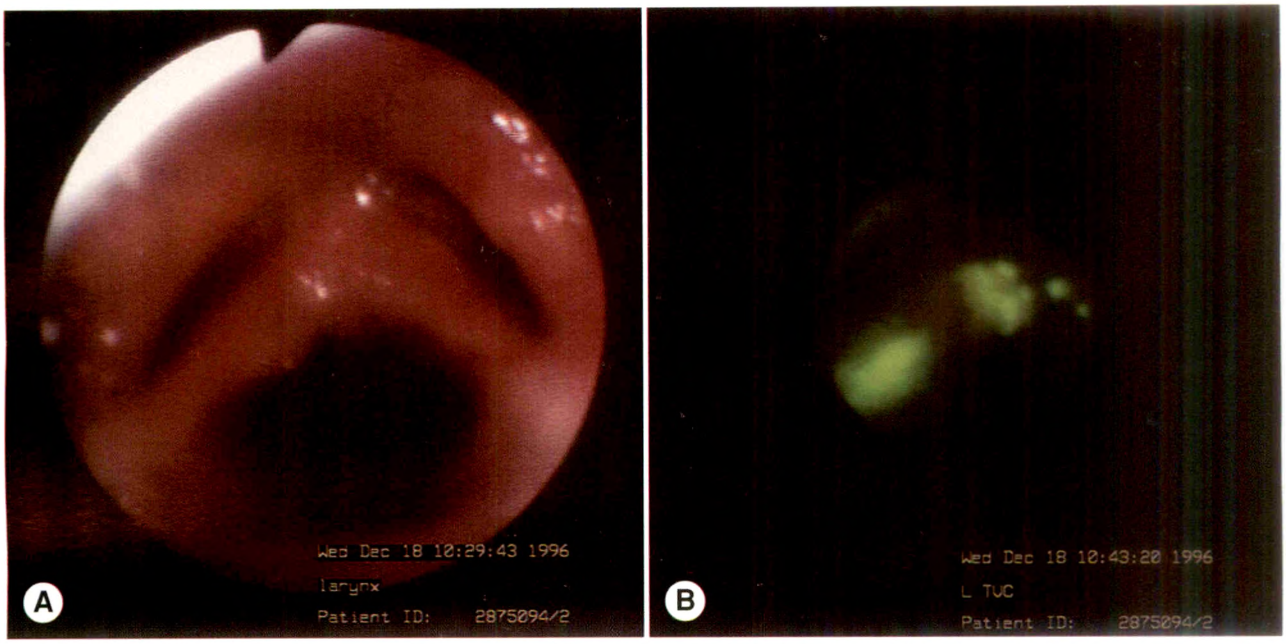


Fig 1. A) White light endoscopy discloses raised lesion in left vocal cord of patient in whom recurrent squamous papilloma was suspected after surgical resection. Image was classified as grade 3 category. B) Fluorescence image of same spot displays focal dark area with clustered brown dots. This was rated as grade 5 image. Lesion was confirmed by biopsy as severe dysplasia with carcinoma in situ.

spectively. Under WLE, the lesions were divided into 5 categories or grades: ulcerative growth with irregular mucosa (grade 5), mass that was suggestive of growth (grade 4), raised lesion with or without color change (grade 3; see example in Fig 1A), smooth mucosa with color change (grade 2), and normal (grade 1). In a few cases, the diseases developed microscopically, and at these sites the mucosal surfaces appeared normal (Fig 2A). With LIFE, normal mucosa was generally visualized as a green image. Dark shadows represented grooves between folds or tubal openings or air passages. In Fig 3, images depicting a normal Rosenmüller fossa in the nasopharynx are shown under WLE and LIFE.

In areas with malignant change, the fluorescence image appeared either as clustered brown dots (Fig 1B) or as a focal dark area with (Fig 2B) or without

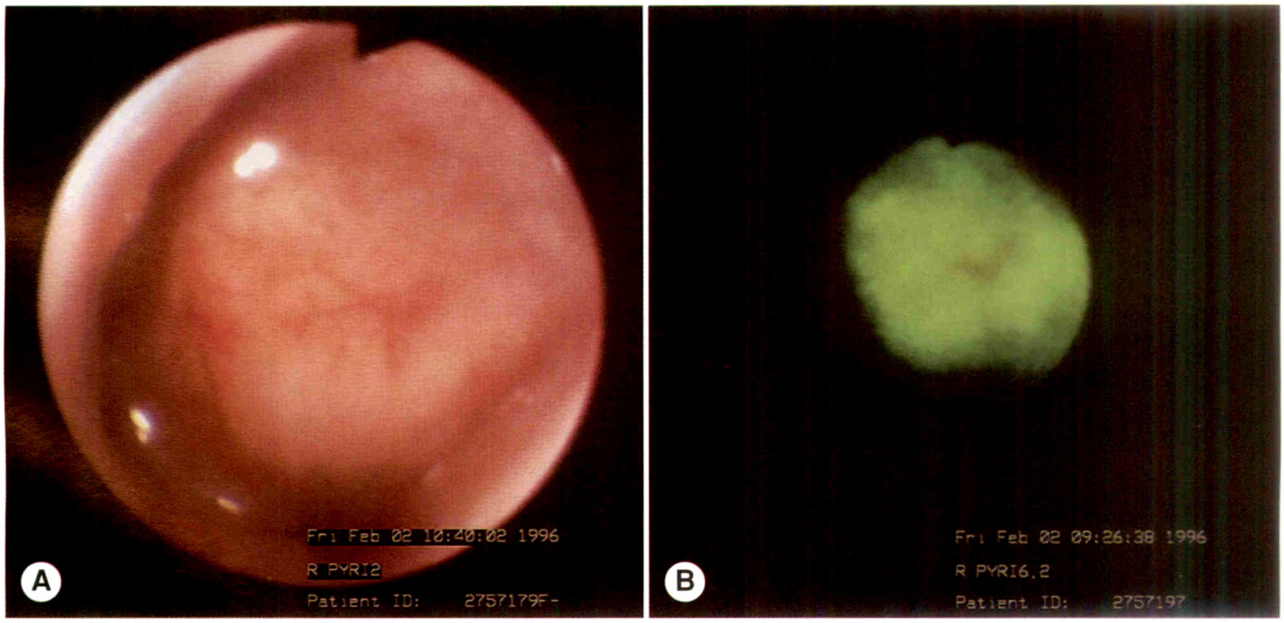


Fig 2. A) White light image suggests grade 1 image of right pyriform sinus in patient with unknown primary cancer. B) Fluorescence investigation of same structure reveals grade 5 image, ie, area with focal brown spot. Suspicious area was diagnosed by histopathology as squamous cell carcinoma.

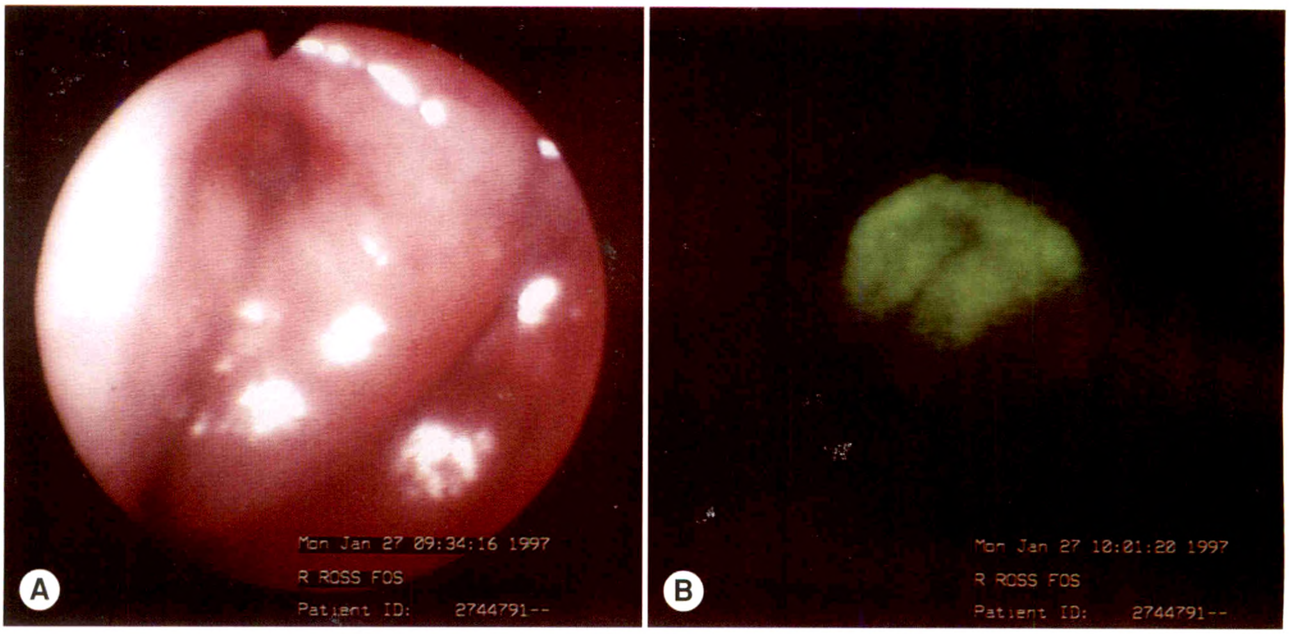


Fig 3. A) White light image demonstrates normal Rosenmüller fossa of patient with unknown primary cancer. Pictorial record was classified as grade 1 image. B) Fluorescence image reveals green mucosa and dark shadow corresponding to grooves between longitudinal folds and opening of eustachian tube. Biopsy findings suggested normal mucosa.

rusty red dots.

In areas with inflamed tissues, blood derived from trauma or contained in granulation tissue strongly absorbed the blue light from the helium-cadmium laser and rendered bright red images, in contrast to the rusty red or brownish color images of the cancerous tissues (clinical trial manual, Xillix Technologies Corp, Vancouver, Canada). Images of the dysplastic lesions most often looked like those of the neoplastic foci, except for a few cases that had typical fluorescence patterns, ie, diffuse dark areas with or without clustered brown-red dots, which were indistinguishable from either normal or inflamed tissues in some locations, eg, base of tongue and nasopharynx.

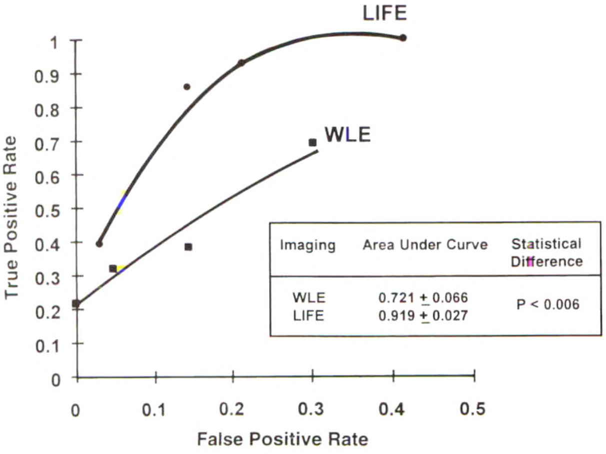


Fig 4. Receiver operating characteristic curves comparing performances of white light endoscopy (WLE) and laser-induced fluorescence endoscopy (LIFE) in detection of head and neck cancers.

Image Rating and ROC Analysis. From the spectrum of morphological changes, as well as the fluorescence changes observed in this study, both the white light and fluorescence images were rated into 5 categories or grades (Table 1). For each grade, a likelihood ratio in relation to likelihood of malignancy was calculated: a higher likelihood ratio implied a higher likelihood of malignancy, etc (Table 1).

The performances of WLE and LIFE were also assessed by analysis of ROC curves, which indicated the superiority of LIFE to WLE by the significant difference between the areas under the curves (Fig 4).

The cutoff points for positive and negative findings were set at the high-sensitivity end of the ROC curves to favor disease discovery. For WLE imaging, normal-appearing mucosa was regarded as a neg-

TABLE 2. PERFORMANCE CHARACTERISTICS OF WHITE LIGHT AND LASER IN PHOTODETECTION OF HEAD AND NECK CANCERS

Test Parameter	*WLE*	*LIFE*	*Combined*
Sensitivity (%)	67.86	92.86	100.00
Specificity (%)	70.00	78.57	60.00
Positive likelihood ratio* (disease presence)	2.26	4.34	2.50
Negative likelihood ratio† (disease absence)	0.46	0.09	0.00
Accuracy (%)	69.39	82.65	71.43

*Positive test outcome more likely in presence of malignancy than in absence of it.

†Negative test outcome less likely in presence of malignancy than in absence of it.

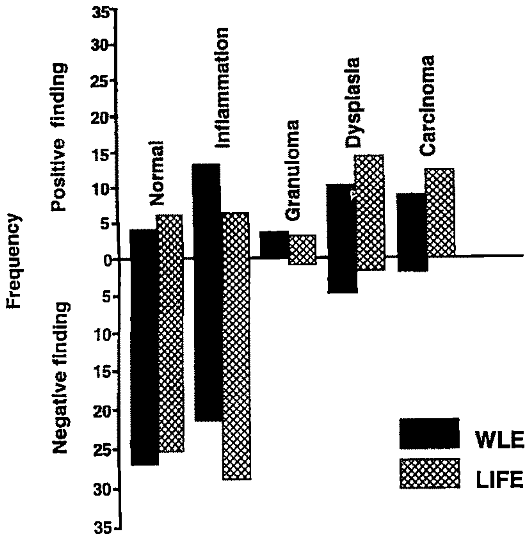


Fig 5. Distributions of positive and negative findings in normal, inflamed, dysplastic, and neoplastic tissues observed by WLE and LIFE.

ative finding in order to achieve a detection sensitivity of 67.86% while sacrificing specificity at 70.00% (Table 2). In LIFE investigation, the cutoff line was drawn above fluorescence grades 1 and 2 to exclude images showing diffuse dark areas and a green area from positive readings. By this criterion, LIFE could identify suspicious sites with a detection sensitivity of 92.86% and a specificity of 78.57% (Table 2).

On an overall basis, the reliability of the two imaging techniques was influenced by false findings. The most serious mistake was the failure to detect the disease while it actually existed. In our study, WLE missed more dysplastic and cancerous lesions than LIFE did (Fig 5). Both methods overdiagnosed most cases of granulomas, some inflammatory foci, and quite a few normal sites as abnormalities. When the results from the two tests were combined (positive finding when either one of the tests yielded a positive result), a slight improvement in sensitivity was obtained, with some loss of specificity (Table 2).

Validity of LIFE Examination in Different Anatomic Sites. The detection sensitivity of LIFE was observed to vary from site to site (Table 3). We found that LIFE was most reliable and sensitive in identifying lesions in the oropharynx. High detection rates accompanied by high false-positive rates were encountered in the examination of structures in the oral cavity, hypopharynx, and larynx. However, an absence of false-negative findings made tumor detection in the oral cavity more reliable than that in the other sites. Among all the sites investigated, LIFE was the least efficient and least reliable in searching for cancers in the nasopharynx, as the test was 33.3% false-negative and 25% false-positive. Nevertheless, one structure in the nasopharynx was relatively free from error: the opening of the eustachian tube.

Interobserver Variability. For widespread use of LIFE, it is important to understand the nature of interobserver bias in image interpretation. Studies were conducted to answer the following questions: 1) How high was the degree of interobserver disagreement on image interpretation? 2) Would the disagreement lead to significant bias? and 3) Would the process of image interpretation require great expertise to make a correct judgment? The 7 investigators who performed this study consisted of 1 expert, 2 medical staff, and 4 residents. The expert was an otolaryngologist who had great expertise in endoscopic examination and had performed more than 100 LIFE investigations. Although the medical staff had expertise in endoscopic examination similar to that of the expert, they had never conducted any LIFE investigation. The residents were trainees in otolaryngology with experience in conventional WLE but without experience in LIFE investigation. Nevertheless, all of the participants were familiar with the theory of tumor imaging by LIF spectroscopy. Before image reading, the expert explained to the other investigators the nature of the 5 grades of fluorescence findings.

In Table 4, results of image readings for a variety of nonmalignant and malignant conditions are sum-

TABLE 3. RELIABILITY OF LIFE SYSTEM IN DETECTION OF PREMALIGNANT AND MALIGNANT LESIONS ARISING FROM DIFFERENT SITES IN HEAD AND NECK

			Detection Rate				
*Site**	*Sample Size*	*Prevalence*	*True Positive*	*False Positive*	*True Negative*	*False Negative*	*Accuracy (%)*
Oral cavity	19	0.421	1.000	0.273	0.727	0.000	84.21
Nasopharynx	19	0.105	0.667	0.250	0.750	0.333	73.68
Oropharynx	32	0.188	1.000	0.038	0.962	0.000	96.88
Hypopharynx and larynx	28	0.357	0.909	0.412	0.588	0.091	71.43
All	98	0.265	0.929	0.214	0.786	0.071	82.65

*Oral cavity is defined as buccal mucosa, floor of mouth, tongue, palate, and retromolar trigone; nasopharynx is defined as nasopharynx and opening of eustachian tube; oropharynx is defined as oropharynx, tongue base, and tonsil; and hypopharynx and larynx are defined as supraglottis, larynx, and pyriform sinus.

TABLE 4. INTEROBSERVER AGREEMENT IN INTERPRETATION OF FLUORESCENCE IMAGES

	*Frequency of Positive Finding**						
Observer	*Normal (n = 8)*	*Inflammation (n = 6)*	*Granuloma (n = 3)*	*Dysplasia (n = 4)*	*Carcinoma (n = 5)*	κ†	*Bias Index and* χ^2 *Test*
Expert	1	2	2	4	5		
Staff 1	2	4	3	4	5	0.525	0.67 ($p > .1$)
Staff 2	2	3	3	4	5	0.606	0.60 ($p > .1$)
Resident 1	2	1	3	4	5	0.611	0.20 ($p > .5$)
Resident 2	2	2	2	4	5	0.687	0.50 ($p > .5$)
Resident 3	3	2	2	4	5	0.687	0.50 ($p > .5$)
Resident 4	2	3	3	4	5	0.606	0.60 ($p > .1$)

*Analysis of variance by ranks using Kruskal-Wallis test indicated no significant difference ($p > .1$) in LIFE interpretation among observers.

†Average κ value = 0.620 ± 0.061.

marized. All investigators totally agreed that images of dysplasia and carcinoma were abnormal. The frequency of positive findings was ranked from the least to the most severe tissue abnormalities, and the variance of the rank was calculated and analyzed by a Kruskal-Wallis test.[13] The result revealed nonsignificant intergroup differences ($p > .10$).

The κ values that indicated the degree of interobserver agreement were averaged to obtain a value of 0.62, which suggested a substantial strength of agreement.[11] Nevertheless, the participants tended to over-read some normal images as abnormal. McNemar bias indices that reflected the degree of disagreement between each investigator and the expert were calculated. A χ^2 test suggested that none of the McNemar bias indices were large enough to support the hypothesis of significant disagreement.[11,13]

DISCUSSION

From changes in tissue native fluorophores and/or alterations in the microscopic architecture of cancer tissues, an important concept in tumor photodetection has arisen.[14-17] It is well recognized that a decrease in green fluorescence together with an increase in red fluorescence is the spectral characteristic commonly found in the investigation of cancerous tissues in the gastrointestinal[6,16,17] and upper aerodigestive tracts.[18,19] Thickening of the nonfluorescent epithelial layer,[17] thinning of the submucosal collagen,[16] and a deficiency in flavins[19] are postulated as possible causes of reduced fluorescence. In a less specific manner, absorption of the excitation light by hemoglobin[20] in neovascularized tissue may lead to reduced fluorescent emission. On the other hand, the observation of a red peak may be ascribed to the presence of endogenous porphyrin, which may possibly be related to altered metabolism of the cancerous tissue[21] or microbial synthesis.[22]

In a lung study, fluorescent images are divided into 3 classes: class I, normal, representing green fluorescence in association with normal anatomic structures; class II, abnormal, being described as diffuse multifocal dark spots or subtle brown areas that do not follow anatomic landmarks; and class III, suspicious, referring to dysplastic and cancerous foci that appear as definite focal brown or brownish red areas (clinical trial manual, Xillix Technologies Corp). In the head and neck region, fluorescence images were rated into 5 classes ranging from a focal dark area with clustered brown–rusty red dots to a green area with or without scattered rusty red dots (Table 1). The fluorescence patterns of the head and neck were slightly different from those of the lung, perhaps because of anatomic and biological differences between these areas.[2] The fluorescence patterns of all malignant lesions were confined to grades 4 and 5, which covered most dysplastic lesions. Although the likelihood ratios for disease detection were high for these high fluorescence grades, biopsy was needed to differentiate premalignant from malignant lesions, as the methods for patient management varied to some extent.[2]

The sensitivity and reliability of LIFE varied from site to site and seemed to be independent of the level of disease prevalence. Except for the oropharynx, LIFE tended to over-represent normal or inflamed areas in the head and neck as high-grade fluorescence. Bacteria[22] and hyperemia[20] could have accounted for the spurious observations. The more important aspect was the lesser likelihood that LIFE would miss true-positive signals. This feature made LIFE an effective diagnostic tool when high sensitivity was required for disease detection, especially when the prevalence of disease varied between 0.25 and 0.65.[10] In our study, the incidence of malignant disease for the entire aerodigestive tract was 0.265. Among all investigated sites, nasopharyngeal images were the most difficult to interpret because of spurious signals. The nasopharynx was also found to be most frequently inflamed (62.5% chance). Unlike the inflamed tissues from other sites, the affected tissue tended to display high-grade fluorescence. This probably masked the subtle fluorescence change in 1 of the 2 dysplastic lesions in the nasopharynx that was missed in this study.

In every endoscopic procedure, white light investigation was performed before LIFE examination to assess the relevance of WLE, because it was observed in our previous study[7] that WLE was required in order to provide the kind of anatomic details that LIFE was unable to obtain. Because WLE was less efficient than LIFE in the detection of subtle changes at the microscopic level, it is unwise to combine WLE and LIFE findings in diagnosis, because the combined effort resulted only in a slight improvement in sensitivity but an important loss of specificity.

In contrast to contact fluorometry, LIFE exploited spectral readings at 2 specified wavelengths (red and green spectra) in the reconstruction of a pseudocolor image to delineate normal and abnormal areas that were easily interpretable. In this study, investigators who had no practical experience in LIFE investigation could correctly differentiate normal from abnormal fluorescence patterns after having received instruction on how to grade and interpret the fluorescence findings. Although they tended to over-read some normal areas or benign conditions as abnormal, the interobserver variation was not large enough to cause significant bias. However, we would anticipate greater variation if these investigators were asked to conduct their own investigations. Every beginner experienced difficulty in locating organs, particularly the less well-defined structures or those without anatomic landmarks, such as the pyriform sinus and nasopharynx. Two major problems or hindrances were 1) the small field of view related to the shallow field depth (not greater than 1.5 cm) and 2) the motility of organs caused by either swallowing or respiratory movement. Therefore, improvement in the efficiency of the light illumination and collection system, as well as enhancement of the speed of data aquisition and image reconstruction, would greatly boost the detection power of LIFE in diagnosis of head and neck cancers.

CONCLUSION

The patterns of tissue fluorescence found in the head and neck were somewhat different from those found in the lung. Originally designed for lung study, LIFE could effectively detect premalignant and malignant lesions in the head and neck, as well. Although LIFE was highly efficient in sensing the subtle changes in early stages of malignancy, it still needed WLE to provide more anatomic details. We found LIFE to be a good diagnostic test, rather than a confirmatory test, because of its high sensitivity (92.86%) and moderate specificity (78.57%). When the criteria for image grading were clearly defined, image interpretation was least affected by interobserver bias. From the information gathered from this study, guidelines for test standardization and for unbiased interpretation of LIFE findings will be proposed, and these may lead to the widespread use of LIFE in detection of head and neck cancers in the near future.

ACKNOWLEDGMENTS — The authors gratefully acknowledge the staff and residents of the Department of Otolaryngology, Ramathibodi Hospital, Mahidol University, for their participation in image interpretation. Sincere thanks are extended to Dr Kanit Muntarbhorn for his help in rewriting the manuscript.

REFERENCES

1. Papadimitrakopoulou VA, Shin DM, Hong WK. Molecular and cellular biomarkers for field cancerization and multistep process in head and neck tumorigenesis. Cancer Metastasis Rev 1996;15:53-76.

2. Wolf GT, Lippman SM, Laramore GE, Hong WK. Neoplasms of the head and neck. In: Holland JF, Ill EF, Bast RC, Kufe DW, Morton DL, Weichselbaum RR, eds. Cancer medicine. 3rd ed. Vol 2. Philadelphia, Pa: Lea & Febiger, 1993:1211-75.

3. Cothren RM, Richards-Kortum R, Sivak MV, et al. Gastrointestinal tissue diagnosis by laser-induced fluorescence spectroscopy at endoscopy. Gastrointest Endosc 1990;36:105-11.

4. Lam S, MacAulay C, Hung J, LeRiche J, Profio AE, Palcic B. Detection of dysplasia and carcinoma in situ with a lung imaging endoscope device. J Thorac Cardiovasc Surg 1993;105:1035-40.

5. Ramanujam N, Mitchell MF, Mahadevan A, Thomsen S, Silva E, Richard-Kortum R. Fluorescence spectroscopy: a diagnostic tool for cervical intraepithelial neoplasia (CIN). Gynecol Oncol 1994;52:31-8.

6. Panjehpour M, Overholt BF, Vo-Dinh T, Haggitt RC, Edwards DH, Buckley FP. Endoscopic fluorescence detection of high-grade dysplasia in Barrett's esophagus. Gastroenterology 1996;111:93-101.

7. Kulapaditharom B, Boonkitticharoen V. Laser-induced fluorescence imaging in localization of head and neck cancers. Ann Otol Rhinol Laryngol 1998;107:241-6.

8. Kluftinger AM, Davis NL, Quenville NF, Lam S, Hung J, Palcic B. Detection of squamous cell cancer and precancerous lesions by imaging of tissue autofluorescence in the hamster cheek pouch model. Surg Oncol 1992;1:183-8.

9. Palcic B, Lam S, Hung J, MacAulay C. Detection and localization of early lung cancer by imaging techniques. Chest 1991;99:742-3.

10. Sackett DL, Haynes RB, Guyatt GH, Tugwell P. Clinical epidemiology: a basic science for clinical medicine. 2nd ed. Boston, Mass: Little, Brown, 1991.

11. Feinstein AR. Clinical epidemiology: the architecture of clinical research. Philadelphia, Pa: WB Saunders, 1985.

12. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982;143:29-36.

13. Zar JH. Biostatistical analysis. London, England: Prentice-Hall, 1974.

14. Alfano RR, Das BB. Light sheds light on cancer. Distinguishing malignant tumors from benign tissues and tumors. Bull NY Acad Med 1991;67:143-50.

15. Qu J, MacAulay C, Lam S, Palcic B. Optical properties of normal and carcinoma bronchial tissue. Appl Opt 1994;33: 7397-405.

16. Romer TJ, Fitzmaurice M, Cothren RM, et al. Laser-induced fluorescence microscopy of normal colon and dysplasia in colonic adenomas: implications for spectroscopic diagnosis. Am J Gastroenterol 1995;90:81-7.

17. Bottroli G, Croce AC, Locatelli D, et al. Natural fluorescence of normal and neoplastic human colon: a comprehensive "ex vivo" study. Lasers Surg Med 1995;16:48-60.

18. Yang Y, Ye Y, Li F, Li Y, Ma P. Characteristic autofluorescence for cancer diagnosis and its origin. Lasers Surg Med 1987;7:528-32.

19. Silberberg MB, Savage HE, Tang GC, Sacks PG, Alfano RR, Schantz SP. Detecting retinoic acid–induced biochemical alterations in squamous cell carcinoma using intrinsic fluorescence spectroscopy. Laryngoscope 1994;104:278-82.

20. Liu CH, Tang GC, Pradhan A, Sha WL, Alfano RR. Effects of self-absorption by hemoglobins on the fluorescence spectra from normal and cancerous tissues. Lasers Life Sci 1989; 8:167-76.

21. Leibovici L, Schoenfeld N, Yehoshua HA, et al. Activity of porphobilinogen deaminase in peripheral blood mononuclear cells of patients with metastatic cancer. Cancer 1988;62:2297-300.

22. Harris DM, Werkhaven J. Endogenous porphyrin fluorescence in tumors. Lasers Surg Med 1987;7:467-72.

≈

INTERNATIONAL HEARING AID CONFERENCE

International Hearing Aid Conference VI. Novel Processing and Fitting Strategies will be held in Iowa City, Iowa, June 7-9, 2001. For information, see web site www.medicine.uiowa.edu/otolaryngology/news/news, or contact Rich Tyler by telephone (319-356-2471) or e-mail (rich-tyler@uiowa.edu).

Ann Otol Rhinol Laryngol 110:2001

GLOBOSAL BASAL CELLS ARE IDENTIFIED AS PROLIFERATING CELLS IN MOUSE OLFACTORY EPITHELIUM

YASUSHI OHTA, MD KEIICHI ICHIMURA, MD
TOCHIGI, JAPAN

Although anti-bromodeoxyuridine (BrdU) antibodies are used for detecting proliferating cells, they may stain proliferating cells in phases following the S-phase, except for G1. Anti-Ki67 antibodies are widely used for detecting proliferating cells in all phases of the cell cycle (G1-, S-, G2-, and M-phase), but not in resting cells (G0-phase). Anti–cyclin D1 antibodies are used to detect proliferating cells in the G1-phase. The present study investigated the olfactory epithelium of mice by double immunostaining using antibodies to BrdU, Ki67, cyclin D1, and cytokeratin 14 (CK14). The cells positive to the anti-BrdU antibody, the anti-Ki67 antibody, and the anti–cyclin D1 antibody differed from the cells positive to the anti-CK14 antibody. Thus, we confirmed that the proliferating cells in all the phases of the cell cycle, including the G1-phase, were globosal basal cells, which were the precursors of the olfactory cells.

KEY WORDS — anti–cyclin D1 antibodies, anti-Ki67 antibodies, globosal basal cells, olfactory epithelium, proliferating cells.

INTRODUCTION

Olfactory cells in the olfactory epithelium are continually renewed throughout life. Proliferation, differentiation, and death of olfactory cells occur continuously, even in adult animals. It has been suggested that the progenitors of the olfactory cells are basal cells.[1] Recent immunohistochemical studies have shown differences between horizontal basal cells (HBCs), which are in direct contact with the basement membrane and which are positive for anti-cytokeratin (CK) antibodies, and globosal basal cells (GBCs), which lie between the layers of the HBCs and olfactory cells and which are devoid of CK.[2,3] Using a combination of the keratin and the bromodeoxyuridine (BrdU) immunohistochemical methods to label proliferating cells showed that the HBCs, which were labeled by the anti-keratin antibody, were not labeled by the anti-BrdU antibody. The GBCs, which were not labeled by the anti-keratin antibody, were labeled by the anti-BrdU antibody. These findings are consistent with the hypothesis that the GBCs are precursors of the olfactory cells.[4,5]

However, the BrdU-positive cells do not reflect all the proliferating cells. Anti-BrdU antibodies may stain the proliferating cells following the S-phase only, but not those in the G1-phase. Anti-Ki67 antibodies are used widely to detect proliferating cells in all phases of the cell cycle (G1-, S-, G2-, and M-phase), but not resting cells (G0-phase).[6] Anti–cyclin D1 antibodies are used to detect proliferating cells that are in the G1-phase.[7]

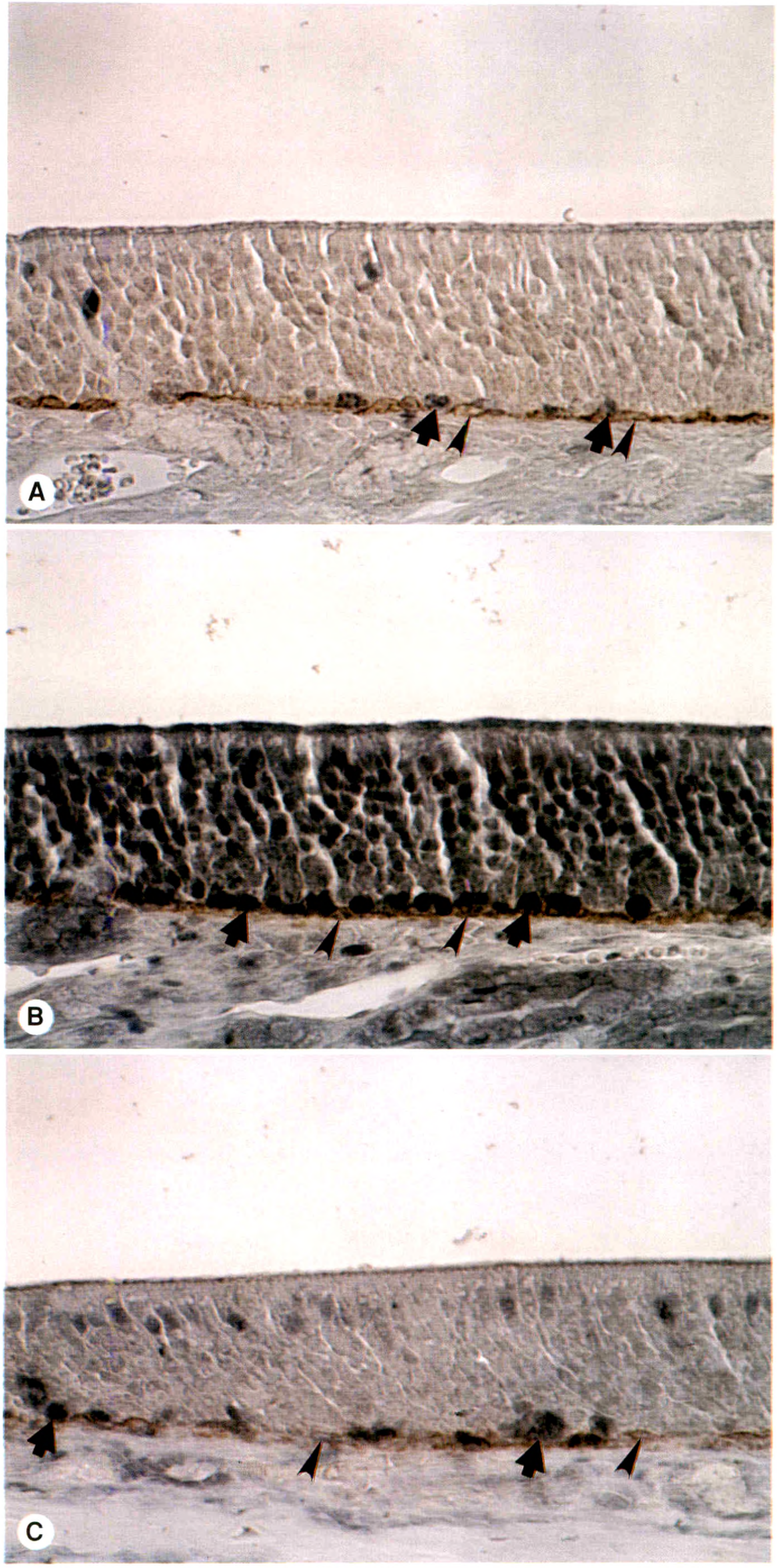
We investigated the olfactory epithelium of mice by double immunostaining using antibodies to BrdU, Ki67, cyclin D1, and CK14. Results were compared between the BrdU-positive cells and the CK14-positive cells, the Ki67-positive cells and the CK14-positive cells, and the cyclin D1–positive cells and the CK14-positive cells.

MATERIALS AND METHODS

We examined 6 adult (postnatal week 6) mice. BrdU was injected into each mouse at 1 to 1.5 hours before fixation. Each mouse was decapitated to obtain specimens, which were decalcified in 10% ethylenediaminetetraacetic acid. The fixed specimens were embedded in paraffin and cut into sections 4 µm thick. After microwave treatment, the sections were incubated for 24 hours at 4°C with anti-CK14 monoclonal IgG (Novocastra Laboratories Ltd, Newcastle, England). Immunohistochemical staining was performed with an LSAB kit (Dako Japan, Kyoto). The immunoreactive antibody-antigen complexes were visualized with 3,3-diaminobenzidine tetrahydrochloride (DAB). Next, the sections were incubated in glycine hydrochloride buffer for 1 hour. They were then incubated for 24 hours at 4°C with the following: anti-BrdU monoclonal IgG (Novocastra Laboratories Ltd), anti-Ki67 polyclonal IgG (Novocastra Laboratories Ltd), and anti–cyclin D1 monoclonal IgG (MBL, Nagoya, Japan). Immunohistochemical staining was performed with an LSAB kit. The immunoreactive antibody-antigen complexes were visualized with DAB-cobalt. After staining, we examined the relationships between the BrdU- and CK14-positive cells, the Ki67- and CK14-positive cells, and the cyclin D1– and CK14-positive cells in the sections.

From the Department of Otolaryngology–Head and Neck Surgery, Jichi Medical School, Tochigi, Japan.

CORRESPONDENCE — Yasushi Ohta, MD, Dept of Otolaryngology–Head and Neck Surgery, Jichi Medical School, Minamikawachi, Tochigi, 329-0498, Japan.



Olfactory epithelia of 6-week-old mice (diaminobenzidine and hematoxylin, original ×200). A) Stained by anti-BrdU monoclonal IgG and anti-CK14 monoclonal IgG. Arrows — BrdU-positive cells; arrowheads — CK14-positive cells. B) Stained by anti-Ki67 polyclonal IgG and anti-CK14 monoclonal IgG. Arrows — Ki67-positive cells; arrowheads — CK14-positive cells. C) Stained by anti–cyclin D1 monoclonal IgG and anti-CK14 monoclonal IgG. Arrows — cyclin D1–positive cells; arrowheads — CK14-positive cells.

RESULTS

The BrdU-, Ki67-, cyclin D1–, and CK14-positive cells were identified abundantly in the basal layers of the olfactory epithelium of 6 mice. The number of Ki67-positive cells was the greatest in 3 kinds of positive cells labeled by anti–proliferating cell antigens. The number of BrdU-positive cells exceeded that of the cyclin D1–positive cells.

The BrdU-positive cells were found to differ from the CK14-positive cells by immunohistochemical double staining. The Ki67-positive cells and the cyclin D1–positive cells also differed from the CK14-positive cells. However, a few of the BrdU-, Ki67-, and cyclin D1–positive cells were identical to the CK14-positive cells (see Figure).

DISCUSSION

CK14 is a cytokeratin intermediate filament protein (50 kd). Because an anti-CK14 antibody is useful for distinguishing basal cells, we used anti-CK14 antibody to detect HBCs.[8] The latter were found to be stained by anti-CK14 antibody.[9]

Recent immunohistochemical studies that used a combination of the keratin and BrdU immunohistochemical methods have shown that HBCs, which were labeled by the anti-keratin antibody, were not labeled by the anti-BrdU antibody, whereas GBCs, which were not labeled by the anti-keratin antibody, were labeled by the anti-BrdU antibody.[4,5]

We obtained the same results in the present study (see Figure, A). These findings are consistent with the hypothesis that the precursors of the olfactory cells are GBCs. However, anti-BrdU antibodies may stain proliferating cells following the S-phase, but not in the G1-phase, because BrdU can be introduced into the proliferating cells that incorporate BrdU into DNA during the S-phase before cell division. Accordingly, an anti-BrdU antibody might not stain all proliferating cells in the olfactory epithelium.

Anti-Ki67 antibodies are used widely for detecting proliferating cells in all phases of the cell cycle (G1-, S-, G2-, and M-phase), but not resting cells (G0-phase).[6] Our studies showed a greater number of Ki67-positive cells than of BrdU-positive cells. The Ki67-positive cells were found to differ from the CK14- positive cells when they were examined by a combination of the immunohistochemical CK14 and Ki67 methods. We concluded that nearly all the proliferating cells, which are the progenitors of olfactory cells, were GBCs.

Cyclin D1 is a protein that acts during the G1-phase of the cell cycle. The maximal expression of cyclin D1 occurs at a critical point in the middle to late G1-phase. For this reason, anti–cyclin D1 antibodies are used to detect proliferating cells in the G1-phase.[7] Our studies showed a slightly smaller number of cyclin D1–positive cells than of BrdU-positive cells. The cyclin D1–positive cells differed from the CK14-positive cells when examined by the combined use of the CK14 and cyclin D1 methods. We concluded that most of the proliferating cells in the G1-phase were GBCs.

The present study confirmed that the proliferating cells in all the phases of the cell cycle, including the G1-phase, were GBCs, the precursors of olfactory cells.

REFERENCES

1. Costanzo RM, Graziadei PP. Development and plasticity of the olfactory system. In: Finger TE, Silver WL, eds. Neurobiology of taste and smell. New York, NY: Wiley, 1987:233-50.
2. Key B, Akeson RA. Immunochemical markers for the frog olfactory neuroepithelium. Dev Brain Res 1990;57:103-17.
3. Suzuki Y, Takeda M. Keratins in the developing olfactory epithelium. Dev Brain Res 1991;59:171-8.
4. Suzuki Y, Takeda M. Basal cells in the mouse olfactory epithelium after axotomy: immunohistochemical and electron-microscopic studies. Cell Tissue Res 1991;266:239-45.
5. Suzuki Y, Takeda M. Basal cells in the mouse olfactory epithelium during development: immunohistochemical and electron-microscopic studies. Dev Brain Res 1993;73:107-13.
6. Gerdes J, Schwab U, Lemke H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983;31:13-20.
7. Inohara S, Kitano Y. Immunohistochemical detection of cyclin D and cyclin A in human hyperproliferative epidermis. Arch Dermatol Res 1994;286:504-6.
8. Westzels RHW, Holland R, Van Haelst UJGM. Detection of basement membrane components and basal cell keratin 14 in noninvasive and invasive carcinomas of the breast. Am J Pathol 1989;134:571-9.
9. Holbrook EH, Szumowsky KEM, Schwob JE. An immunochemical, ultrastructural, and developmental characterization of the horizontal basal cells of rat olfactory epithelium. J Comp Neurol 1995;363:129-46.

Ann Otol Rhinol Laryngol 110:2001

EFFECTS OF TRANSGLOTTAL PRESSURE ON FUNDAMENTAL FREQUENCY OF PHONATION: STUDY WITH A RUBBER MODEL

HIDEYUKI KATAOKA, MD
KAZUTOMO KITAJIMA, MD
SHIGEHIRO OWAKI, MD
OTSU, JAPAN

The fundamental frequency of phonation (F0) change per unit change in transglottal pressure (dF/dP) was studied at different extension lengths and masses of the vibrating part of a rubber model. The dF/dP was decreased when the vibrating part was extended, and also when the mass of the vibrating part was increased. In the former, the F0 was increased, and in the latter, the F0 was decreased. In humans, we have noticed that when the F0 was increased, the dF/dP first decreased and then increased, taking a V-shaped curve as a function of the F0. The present study reveals the mechanism underlying the V-shaped curve observed in humans.

KEY WORDS — fundamental frequency, model, transglottal pressure.

INTRODUCTION

The fundamental frequency of phonation (F0) is influenced by transglottal pressure. We previously investigated the magnitude of F0 change per unit change in transglottal pressure (dF/dP) in humans, and we concluded that the relationship between dF/dP and F0 is not uniform. With dF/dP plotted as a function of F0, the characteristic curve is shaped like the letter V (Fig 1[1]). In the present study, we investigated the mechanism that underlies this nonlinear relationship.

The influence of transglottal pressure on F0 has been analyzed theoretically and experimentally by Titze.[2] He reported that the dF/dP is determined by the degree of stretching or stress of the vocal folds. When the vocal folds are stretched, the dF/dP gets smaller as the F0 gets higher. The left half of the V (the descending limb) reflects this relationship. However, this relationship cannot account for the right half of the V (the ascending limb), because the dF/dP increases with increases in F0. Clearly, additional factors are involved. Titze[2] has proposed that the dF/dP might increase when the vibration is limited to the edge of the vocal folds, as in the falsetto voice.

Observations of the human larynx have indicated that the length of the vocal fold increases with an increasing F0, exhibiting a plateau at high frequencies.[3-5] Hollien[3] reported that the thickness of the vocal folds systematically decreases as the F0 increases. These findings, in the light of the proposal of Titze,[2] suggest that the effect of the width of the vocal folds on the dF/dP might be responsible for the right half of the V-shaped curve. It was assumed that the width of vibration could be expressed in terms of mass (grams). The relationship between dF/dP and mass was investigated with use of a rubber model to elucidate the mechanism underlying the nonlinear, V-

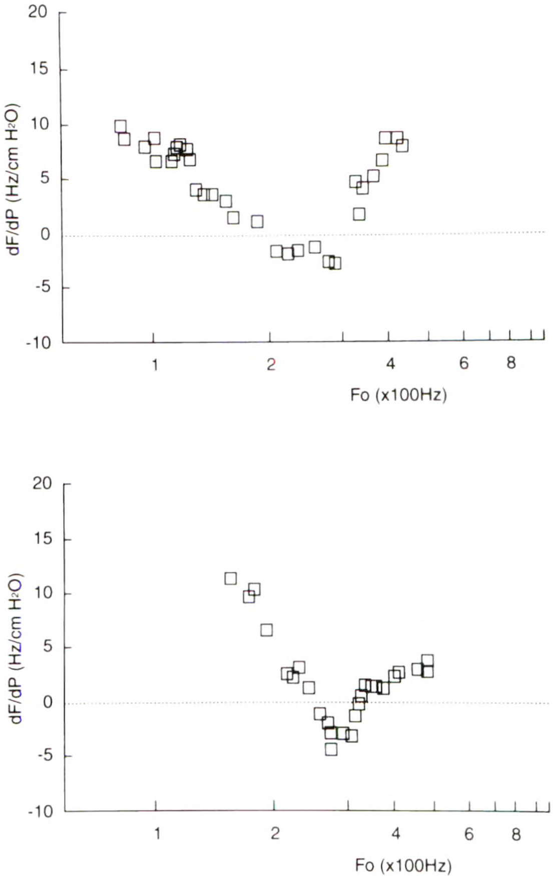


Fig 1. Changes in fundamental frequency (F0) per unit change in transglottal pressure (dF/dP), plotted as function of F0 in 30-year-old man (top) and 28-year-old woman (bottom). Note V shape of curves. (Data from Tanaka et al.[1])

From the Department of Otolaryngology, Shiga University of Medical Science, Otsu, Japan.
CORRESPONDENCE — Kazutomo Kitajima, MD, Dept of Otolaryngology, Shiga University of Medical Science, Seta, Otsu, 520-2192 Japan.

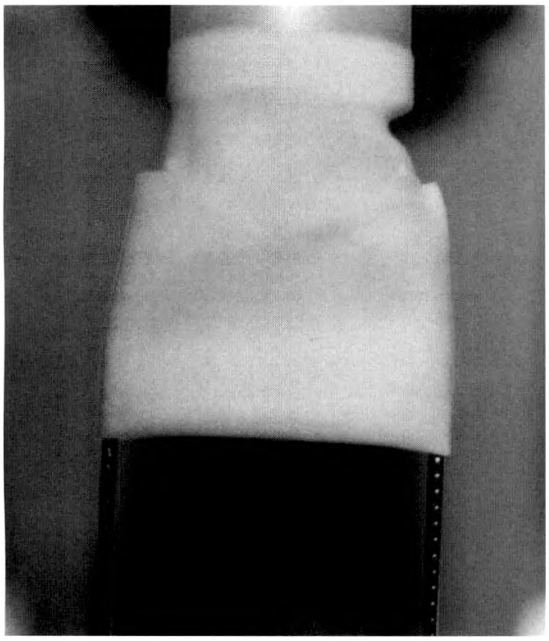


Fig 2. Bottom of rubber tube is expanded by 2 supporting needles.

shaped curve that describes dF/dP as a function of F0.

MATERIALS AND METHODS

The rubber model of the vocal fold was constructed from a finger of a surgical glove. The cut end of the thin rubber tube was folded back on itself. The upper end of the rubber tube was attached to an air supply via a connector with a 19.5-mm outer diameter. At the bottom end of the model, 2 needles with diameters of 0.5 mm (supporting needles) were inserted into the tube, creating 2 pockets (Fig 2). The pockets were open to the atmosphere. The air pressure in the tube was monitored with a pressure transducer (Fig 3). The sound signal and air pressure inside the tube were simultaneously fed into a data recorder. The model vibrated as air was insufflated into the tube. The vibration was recorded on videotape with the aid of stroboscopic light. While the model was vibrating regularly, the air pressure was abruptly reduced by opening a 4-mm (outer diameter) tube attached to the air supply connector (pressure reduction side tube shown in Fig 3), thereby abruptly reducing the F0. The effects of a sudden reduction in air pressure

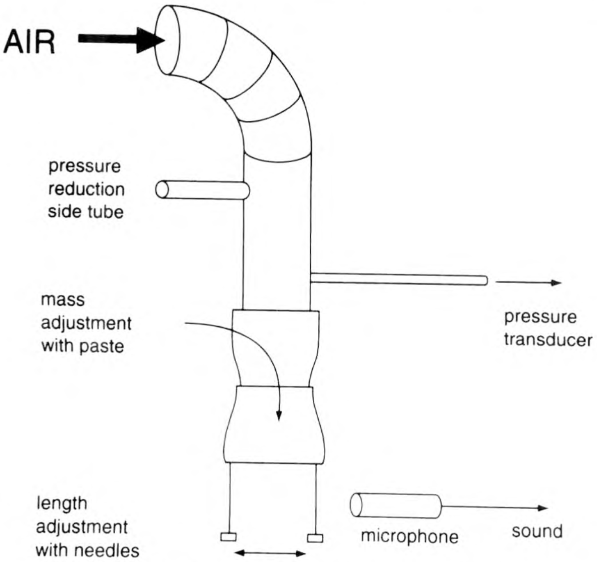

AIR
pressure reduction side tube
mass adjustment with paste
pressure transducer
length adjustment with needles
microphone
sound

Fig 3. Block diagram of experiments.

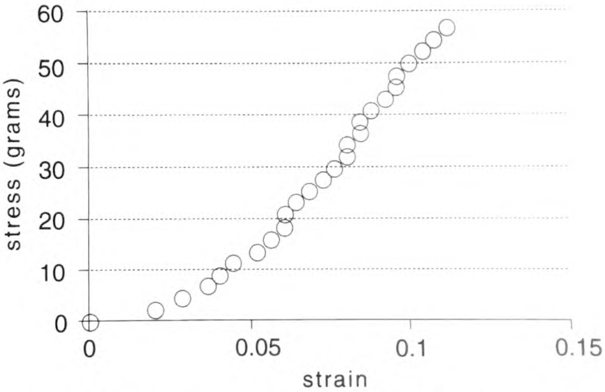


Fig 4. Stress-strain curve of rubber model. Stress (grams) is plotted as function of strain (ratio of extended length to original length). Stress increased almost linearly with elongation.

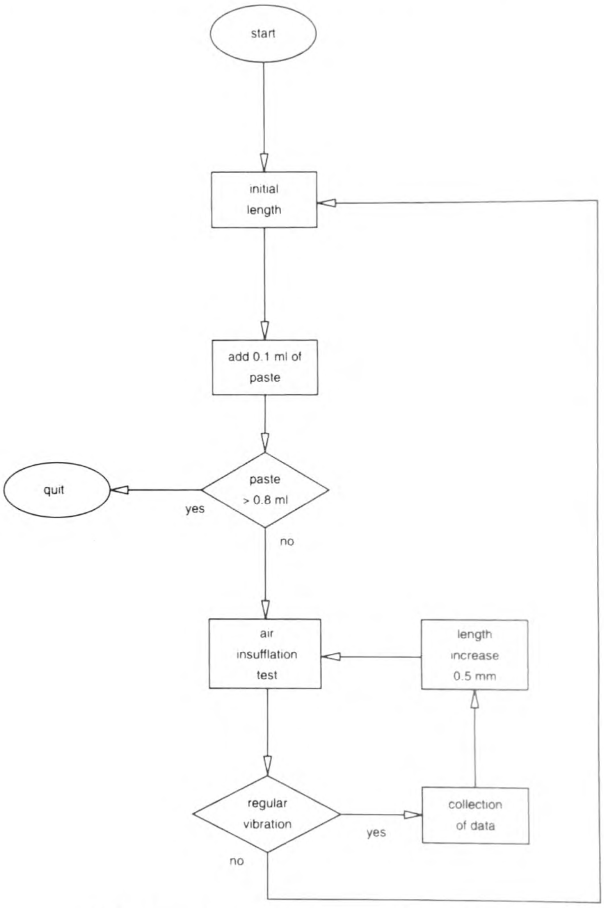


Fig 5. Flowchart of experimental procedures.

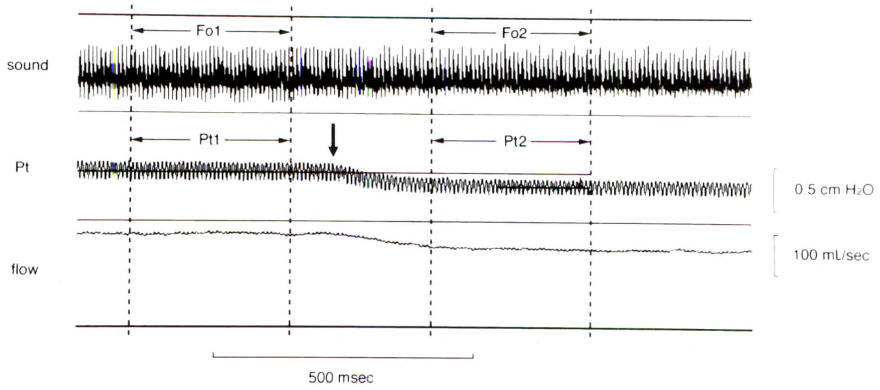


Fig 6. Data displayed on computer screen. Sound wave (top), tube pressure (Pt; middle), and flow (bottom) were used for calculation of dF/dP.

on the F0 were investigated under different conditions of stress and mass with the rubber model.

Incremental Changes in Mass. The mass of the vibrating portion was increased by placing paste (Therasol; WR Medical Electronics Co, Stillwater, Minn) in the pockets at the bottom of the model. The specific gravity of the paste was 1.1607. The incremental changes in mass were expressed in terms of paste volume (milliliters) for simplicity.

Incremental Changes in Length. An incremental increase in length was obtained by increasing the distance (millimeters) between the 2 supporting needles. In a preliminary test, the stress (grams) was examined as a function of elongation (millimeters) by increasing the distance between the 2 needles (Fig 4). Stress increased almost linearly with elongation ($R^2 = .979$; $p < .001$).

It is possible that the mass of the paste further elongated the tube. However, because the pockets at the bottom of the model were supported and expanded by 2 needles, the additional length would be too small to have any appreciable effect.

Study Design. The initial length was 25.1 mm. There was no paste in the pockets at the initial length. With a 1-mL syringe, 0.05 mL of paste was injected into each pocket (0.1 mL total; Fig 5). If the accumulated volume did not exceed 0.8 mL, the air insufflation test was performed. When regular vibration was observed, data were collected. Then, the length was increased by 0.5 mm. This was followed by the air insufflation test and data recording. The length was increased in 0.5-mm steps until no vibrations were observed. When vibrations were not observed, the length was reduced to 25.1 mm. Then, another 0.1 mL of paste was injected into the pockets. At each point, the air insufflation test was performed and data were recorded. The maximum paste volume was 0.8 mL.

Calculation of dF/dP. The dF/dP was calculated from the data display (Fig 6). Before and after each reduction in tube pressure (Pt), the F0 was measured. The dF/dP was calculated as follows: $dF/dP = (F0_1 - F0_2)/(Pt_1 - Pt_2)$, where $F0_1$ (hertz) is the F0 measured before the reduction in Pt calculated in a 300-ms window, $F0_2$ (hertz) is the F0 measured after the reduction in Pt, Pt_1 (centimeters of water) is the average Pt measured before the reduction in the 300-ms window, and Pt_2 (centimeters of water) is the Pt measured after reduction.

Because the airflow was released to the atmosphere at the bottom of the model, $Pt_1 - Pt_2$ could be considered the pressure reduction in the vibrating part, which is similar to the transglottal pressure in hu-

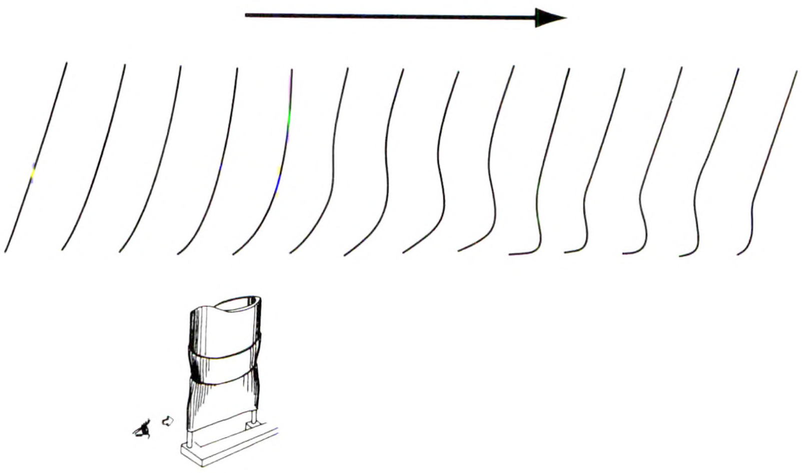


Fig 7. Tracings of right side of wall of rubber model during 1 cycle of vibration (paste volume, 0.6 mL; length, 25.1 mm). Airflow is from top to bottom.

TABLE 1. STUDY RESULTS

Length (mm)	Paste Volume (mL)	F0 (Hz)	dF/dP	Pt1 (cm H_2O)	Flow (mL/s)
25.1	0.1	124.9	37.5	0.87	245.3
25.6	0.1	140.3	26.8	1.02	240.9
26.1	0.1	150.6	32.0	1.21	240.8
26.6	0.1	160.0	8.3	1.38	238.8
27.1	0.1	165.2	26.5	1.54	237.2
27.6	0.1	176.6	18.9	1.68	235.8
28.1	0.1	179.7	16.8	1.86	234.0
28.6	0.1	186.2	28.6	2.04	231.6
25.1	0.2	123.4	23.2	1.11	239.5
25.6	0.2	129.6	28.7	1.34	236.9
26.1	0.2	136.5	23.8	1.52	235.2
26.6	0.2	146.3	10.2	1.69	234.2
27.1	0.2	150.6	15.2	1.81	232.7
27.6	0.2	155.2	15.3	2.04	230.5
28.1	0.2	157.6	15.6	2.26	228.3
28.6	0.2	162.5	9.4	2.36	227.9
25.1	0.3	117.7	19.0	1.27	235.8
25.6	0.3	126.4	15.6	1.53	233.5
26.1	0.3	131.3	13.9	1.68	232.1
26.6	0.3	136.5	17.7	1.81	232.0
27.1	0.3	142.2	16.0	1.95	229.9
27.6	0.3	146.3	12.7	2.23	228.4
28.1	0.3	150.6	14.1	2.45	227.0
28.6	0.3	152.9	9.1	2.62	224.9
25.1	0.4	112.5	17.5	1.48	233.9
25.6	0.4	121.9	12.4	1.69	230.6
26.1	0.4	124.9	17.3	1.82	230.7
26.6	0.4	129.6	14.8	1.95	229.5
27.1	0.4	133.0	14.8	2.06	227.9
27.6	0.4	136.5	16.4	2.21	227.6
28.1	0.4	140.3	10.6	2.41	226.6
28.6	0.4	144.2	10.4	2.43	225.5
25.1	0.5	108.9	14.9	1.48	231.0
25.6	0.5	116.4	13.0	1.72	229.8
26.1	0.5	121.9	12.3	1.84	229.9
26.6	0.5	124.9	12.1	1.91	228.7
27.1	0.5	128.0	7.7	2.08	228.7
27.6	0.5	131.3	12.1	2.22	227.4
28.1	0.5	133.0	11.9	2.37	227.6
28.6	0.5	138.4	9.8	2.52	226.0
25.1	0.6	104.5	17.2	1.51	231.5
25.6	0.6	113.8	12.6	1.83	229.0
26.1	0.6	119.1	12.9	1.96	226.9
26.6	0.6	121.9	10.6	2.09	225.9
27.1	0.6	124.9	9.8	2.25	225.6
27.6	0.6	126.4	12.2	2.30	225.1
28.1	0.6	129.6	10.7	2.48	224.6
25.1	0.7	102.4	15.5	1.58	230.3
25.6	0.7	111.3	11.4	1.82	226.2
26.1	0.7	115.1	13.1	1.98	225.6
26.6	0.7	120.5	10.3	2.17	224.9
27.1	0.7	121.9	10.3	2.26	225.2
27.6	0.7	124.9	11.0	2.39	222.2
25.1	0.8	103.4	13.7	1.69	228.6
25.6	0.8	110.1	10.3	1.84	226.8
26.1	0.8	115.1	9.4	2.09	226.6
26.6	0.8	117.7	10.6	2.18	223.7
27.1	0.8	121.9	8.5	2.36	223.1

F0 — fundamental frequency of phonation; dF/dP — change in F0 per unit change in transglottal pressure; Pt1 — average tube pressure measured before reduction in 300-ms window.

mans. The mean flow rate (milliliters per second) was also obtained during the time interval of Pt1 measurement. The trial in which the vibration was most stable was used for later analysis.

The dF/dP could be obtained by simply calculating the slope of the regression line between the F0 and the Pt1, because there was no laryngeal reflex. A laryngeal reflex would be expected in humans after a sudden change in pressure. A pressure reduction side tube was used to simulate the conditions under which human subjects have been tested.[1]

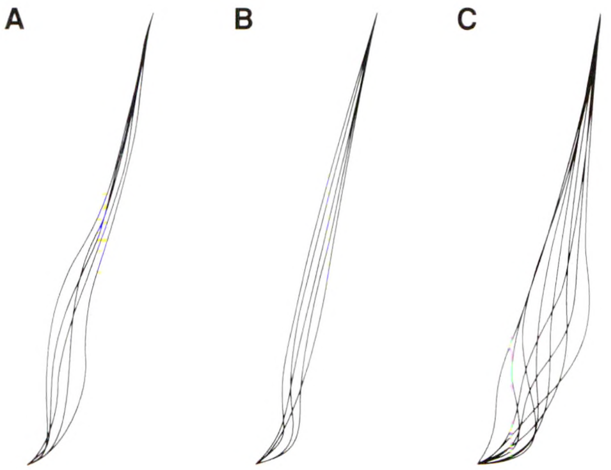
RESULTS

Characteristics of Rubber Model. The extension length (millimeters), paste volume (milliliters), F0 (hertz), dF/dP, Pt (centimeters of water), and flow (milliliters per second) were measured at each point (Table 1). The length was changed from 25.1 mm to 28.6 mm at each paste volume up to 0.5 mL. Thereafter, the change in length was limited, because the vibrations became unstable. The F0 ranged from 102.4 to 186.2 Hz, the Pt1 ranged from 0.87 to 2.62 cm H_2O, and the flow ranged from 222.2 to 245.3 mL.

Correlation coefficients (r) were calculated for the relationships between F0 and length, paste volume, Pt1, and flow. The F0 increased with length (r = .957 to .990, depending on the paste volume). The F0 decreased with paste volume (r = –.942 to –.973, depending on length). The F0 also increased with Pt1 (r = .894 to .993, depending on paste volume and length), except at a paste volume of 0.8 mL. Further, the F0 decreased with flow (r = –.912 to –.981, depending on paste volume and length), except at a length of 28.6 mm.

The expected relationships between F0 and length, paste volume, and Pt were observed. The relationship between flow and F0 was negative. This may have been a result of increased resistance in the vibrating part. The flow range, however, was small. In humans, the airflow does not change in a consistent way as the F0 is altered.[6] Thus, this relationship was not a critical issue in this study.

The vibrating behavior of the model was recorded under different conditions of length and paste volume (Figs 7 and 8). The visual angle for videography was along the straight line connecting the 2 supporting needles. With air insufflation from top to bottom, the rubber wall was expanded to full length. This was followed by a traveling wave from top to bottom (Fig 7). With an increase in length from 25.1 mm (Fig 8A) to 28.1 mm (Fig 8B) at a paste volume of 0.2 mL, vibration was observed from top to bot-



0.2 mL (25.1 mm) 123.4 Hz

0.2 mL (28.1 mm) 157.6 Hz

0.6 mL (25.1 mm) 104.5 Hz

Fig 8. Tracings of wall of rubber model during vibration under 3 different conditions of length and paste volume. A) Paste volume, 0.2 mL; length, 25.1 mm. B) Paste volume, 0.2 mL; length, 28.1 mm. C) Paste volume, 0.6 mL; length, 25.1 mm. Note that rubber wall moves laterally, forming traveling wave from top to bottom.

tom. The amplitude, however, was decreased. With an increase in paste volume from 0.2 mL (Fig 8A) to 0.6 mL (Fig 8C) at a length of 25.1 mm, vibration was observed from top to bottom, with an increase in amplitude.

Vibration was observed from top to bottom under all conditions. Because vibration was observed over the full width between the 2 needles at the bottom of the model (not displayed here), it was postulated that the vibrating area was not influenced by the length or the paste volume. This observation validates this model for investigating the effects of length and paste volume on dF/dP.

TABLE 2. CORRELATION COEFFICIENTS BY LENGTH

Length	*Relationship Between dF/dP and Paste Volume*		*Relationship Between dF/dP and F0*	
(mm)	*r*	*p*	*r*	*p*
25.1	−.850	.0160	.806	.0157
25.6	−.850	.0074	.867	.0053
26.1	−.855	.0068	.935	.0007
26.6	−.071	.8665*	−.185	.6618*
27.1	−.839	.0092	.928	.0009
27.6	−.824	.0227	.840	.0180
28.1	−.922	.0089	.923	.0082
28.6	−.683	.2041*	.856	.0641*

*Not statistically significant ($p > .05$).

Because the tube was folded back on itself, there were 2 layers of rubber. During vibration, these 2 layers seemed to move as 1 wall, although the movements of the inner layer were not studied in detail. The paste was always situated at the bottom of the model, because of gravity, and the traveling wave moved from top to bottom.

Correlations Between dF/dP and Length and Paste Volume. First, correlation coefficients were calculated for the relationships between dF/dP and paste volume and F0 at each length (Table 2). A negative correlation between dF/dP and volume was noted, except at 2 lengths (26.6 mm and 28.6 mm). A positive correlation was noted between dF/dP and F0, except at 26.6 mm and 28.6 mm (Fig 9A).

Then, correlation coefficients were calculated for the relationship between dF/dP and length, as well as that between dF/dP and F0, at each paste volume (Table 3). The dF/dP decreased with length, although meaningful correlations were only observed at 0.2 and 0.3 mL. A negative correlation was noted be-

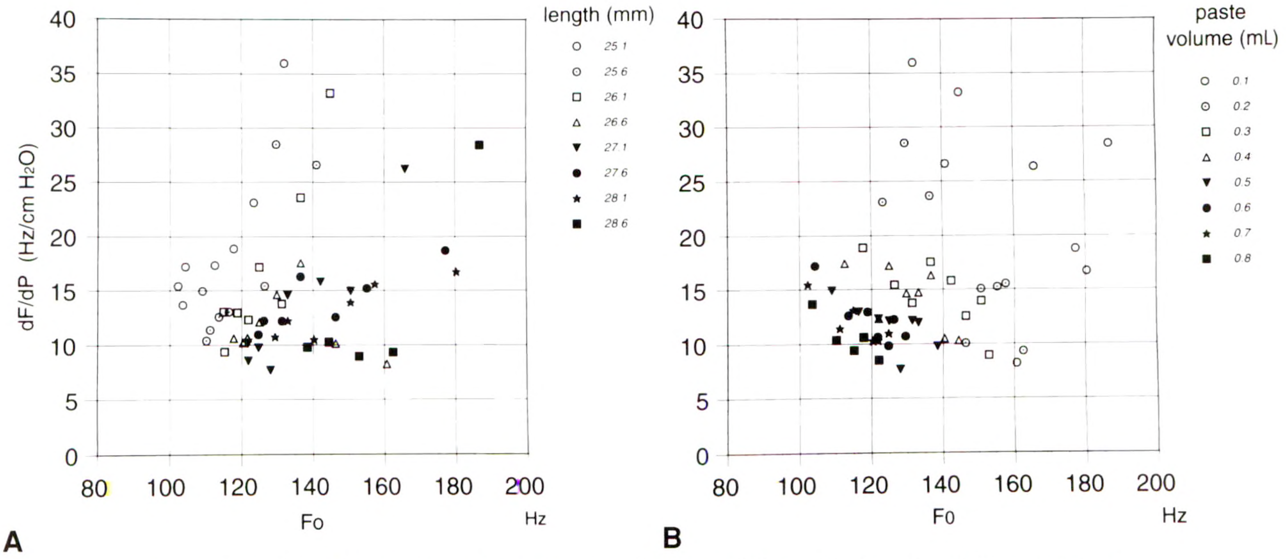


Fig 9. Plots of dF/dP as function of F0 under 8 different conditions of A) length and B) paste volume.

TABLE 3. CORRELATION COEFFICIENTS BY PASTE VOLUME

Paste Volume (mL)	*Relationship Between dF/dP and Length*		*Relationship Between dF/dP and F0*	
	r	*p*	*r*	*p*
0.1	−.419	.3019*	−.512	.1944*
0.2	−.775	.0234	−.830	.0107
0.3	−.778	.0231	−.750	.0320
0.4	−.629	.0945*	−.649	.0814*
0.5	−.628	.0957*	−.685	.0607*
0.6	−.735	.0597*	−.870	.0108
0.7	−.754	.0836*	−.862	.0271
0.8	−.811	.0956*	−.885	.0461

*Not statistically significant ($p > .05$).

tween dF/dP and F0, except at 0.1, 0.4, and 0.5 mL (Fig 9B). Thus, the dF/dP increased when the F0 was raised by a reduction in the mass. The dF/dP decreased when the F0 was raised by elongation.

DISCUSSION

When the dF/dP in humans is plotted as a function of F0, a characteristic V-shaped curve emerges. In humans, the length and mass of the vocal folds change simultaneously when the F0 is raised. From the results of this study, the mechanism underlying the V-shaped curve can be explained. In the F0 range in which the dF/dP is decreasing, elongation plays a dominant role in frequency determination. In the F0 range in which the dF/dP is increasing, mass reduction plays a dominant role in frequency determination. The dominant factor in frequency determination changes from length to mass around 200 to 250 Hz in humans.

If this mechanism was indeed responsible for the V-shaped curve, it could be reproduced by first elongating the model at a fixed volume, followed by a reduction in volume at a fixed length. The curve plotted by means of this construct (Fig 10; 25.1 to 27.1 mm at a volume of 0.8 mL, followed by a reduction in volume to 0.1 mL at 27.1 mm) closely resembles the curve seen in humans (Fig 1).

Contrary to our expectation, the correlation between dF/dP and elongation was weak in most cases. This finding may be explained by the restricted range of elongation at large paste volumes. The F0 range was therefore limited under these conditions. If the F0 range had been extended at larger volumes, more meaningful correlations might have been observed. Further, this study was performed with a cover-only model.[7] In humans, the existence of anatomic structures surrounding the vocal folds, especially the thyroarytenoid muscle, enables the cover to vary over a wider range of stress.[6] In our model, stress was changed only by elongation, representing a small portion of the range of stress exhibited in humans. This difference could also explain the weak correlation between dF/dP and elongation.

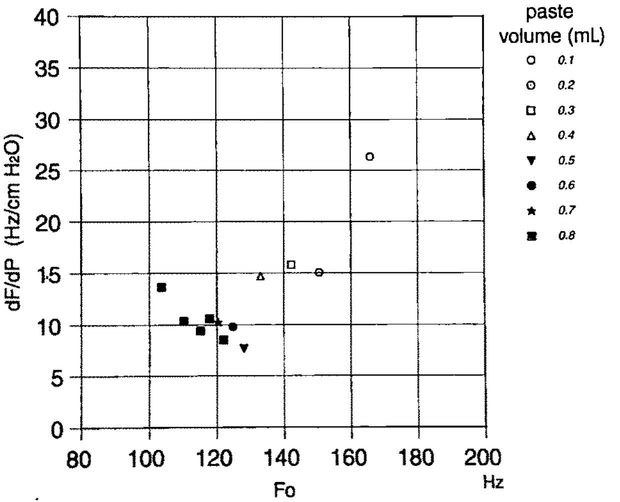


Fig 10. Characteristic V-shaped curve reproduced from selected data. First, length is increased from 25.1 to 27.1 mm at paste volume of 0.8 mL (left half of V). Then, paste volume is reduced to 0.1 mL at length of 27.1 mm (right half of V).

CONCLUSIONS

The dF/dP was studied at different lengths and masses of the vibrating part of the rubber model. The dF/dP was decreased when the vibrating part was elongated, and also when the mass of the vibrating part was increased. In the former, the F0 was increased, and in the latter, the F0 was decreased. In humans, we have noticed that when the F0 was increased, the dF/dP first decreased and then increased, taking a V-shaped curve as a function of F0. The present results explain the mechanism underlying the V-shaped curve. The left half of the V (the descending limb) is created when elongation predominates in F0 determination, and the right half of the V (the ascending limb) is produced when mass reduction predominates in F0 determination.

REFERENCES

1. Tanaka K, Kitajima K, Kataoka H. Effects of transglottal pressure change on fundamental frequency of phonation: preliminary evaluation of the effect of intraoral pressure change. Folia Phoniatr Logop 1997;49:300-7.

2. Titze IR. On the relation between subglottal pressure and fundamental frequency in phonation. J Acoust Soc Am 1989;85:901-6.

3. Hollien H. On vocal registers. J Phonetics 1974;2:125-43.

4. Nishizawa N, Sawashima M, Yonemoto K. Vocal fold

length in vocal pitch change. In: Fujimura O, ed. Vocal physiology: voice production, mechanisms and functions. New York, NY: Raven Press, 1988:75-82.

5. Sonninen A, Hurme O, Vilkman E. Roentgenological observations on vocal fold length-changes with special reference to register transition and open/covered voice. Scand J Log Phon 1992;17:95-106.

6. Baken RJ. Clinical measurement of speech and voice. London, England: Taylor & Francis, 1987:300-1.

7. Fujimura O. Discussion of phonatory models. In: Fujimura O, ed. Vocal physiology: voice production, mechanisms and functions. New York, NY: Raven Press, 1988:259-60.

≈

MANAGEMENT OF THE TINNITUS PATIENT

The Ninth Annual Conference on the Management of the Tinnitus Patient, for professionals and tinnitus patients, will be held in Iowa City, Iowa, October 4-6, 2001. For information, see web site www.medicine.uiowa.edu/otolaryngology/news/news or contact Rich Tyler by telephone (319-356-2471) or e-mail (rich-tyler@uiowa.edu).

≈

NEW FRONTIERS IN THE AMELIORATION OF HEARING LOSS

The international conference New Frontiers in the Amelioration of Hearing Loss will be held in St Louis, Missouri, March 22-25, 2001. For information, contact Sarah Uffman, Central Institute for the Deaf, Department of Research, 4560 Clayton Ave, St Louis, MO 63110; telephone (314) 977-0278; fax (314) 977-0030; e-mail: suffman@cid.wustl.edu.

Ann Otol Rhinol Laryngol 110:2001

ENDOBRONCHIAL TUMORS IN CHILDREN: AN UNCOMMON CLINICAL ENTITY

LT KIRBY J. SCOTT, MC USN
PORTSMOUTH, VIRGINIA
DAVID DARROW, MD, DDS
NORFOLK, VIRGINIA
JOHN H. GREINWALD, JR, MD
CINCINNATI, OHIO
RICHARD J. H. SMITH, MD
IOWA CITY, IOWA

We performed a retrospective chart review of 10 children in whom endobronchial tumors were diagnosed in a tertiary-care children's medical center from 1988 to 1998. Of the 10 patients, 6 were male and 4 were female (8 white, 1 Hispanic, 1 African American). The mean age at presentation was 5.2 years. Eight tumors were benign, and 2 were malignant; 4 were on the right side, 3 were on the left side, and 3 were bilateral. Histologic findings included 3 bronchial papillomas, 3 inflammatory masses, 1 endobronchial hemangioma, 1 leiomyoma, 1 mucoepidermoid carcinoma, and 1 bronchial carcinoid. Endobronchial tumors in children are a rare disorder. The diagnosis requires a high index of suspicion in children with atypical or chronic respiratory complaints. Newer adjuvant medical therapies and surgical innovations offer improved disease control in these patients, and a multidisciplinary approach is often warranted.

KEY WORDS — endobronchial mass, pediatric tumor, tracheal tumor.

INTRODUCTION

Endobronchial tumors in children are exceedingly rare, and for this reason they are often misdiagnosed as more benign conditions, so that definitive treatment is delayed. Fewer than 200 cases have been described, typically in reports of small series,[1-3] usually biased toward bronchial adenomas. Since the early 1980s, there has not been a comprehensive review describing advances in the diagnosis and treatment of these lesions.

We describe 10 new cases of pediatric endobronchial tumors. This report represents the largest single experience with this rare group of heterogeneous tumors. Representative cases highlight both the diagnostic dilemmas and the recent advances in the treatment of these tumors.

SUBJECTS AND METHODS

The medical records of patients treated at the Children's Hospital of Iowa and the Children's Hospital of the King's Daughters from 1988 to 1998 with a diagnosis of an endobronchial mass were reviewed retrospectively. During this period, an endobronchial mass was diagnosed by bronchoscopy in 10 children. Data pertaining to the chief complaint, presentation, treatment, clinical course, and pathological findings were analyzed.

RESULTS

Endobronchial tumors were identified in 10 children (6 male and 4 female) ranging in age from 11 months to 13 years (average age at presentation, 5.2 years; 8 white, 1 Hispanic, 1 African American; see Table). Eight tumors were benign, and 2 were malignant; 3 lesions were found in the left main bronchus, 4 were found in the right main bronchus, and 3 were bilateral. The presenting complaints included recurrent pneumonia or bronchitis in 4 patients, wheezing in 5, cough in 3, dyspnea in 3, and hoarseness in 2. The diagnosis was confirmed by bronchoscopy in all cases, although computed tomography (CT) was used as a diagnostic adjunct in 7 cases.

Surgical intervention consisted of endoscopic resection in 8 patients; in 5 patients, complete resection was possible. Segmental pulmonary wedge resection was completed in the patient with mucoepidermoid carcinoma and in the patient with bronchial carcinoid. The former case is discussed in detail; the latter patient has no evidence of disease 5 years after surgery.

Numerous bronchoscopic resections, with and without the use of a CO2 laser, were required for the 3 patients with papillomatosis of the main bronchi.

From the Department of Otolaryngology–Head and Neck Surgery, Naval Medical Center Portsmouth, Portsmouth (Scott, Greinwald), and the Department of Otolaryngology–Head and Neck Surgery, Children's Hospital of the King's Daughters, Norfolk (Darrow), Virginia, and the Division of Pediatric Otolaryngology, Department of Otolaryngology–Head and Neck Surgery, Children's Hospital of Iowa, Iowa City, Iowa (Smith). The views expressed are those of the authors and do not reflect the official policy or position of the Department of the Navy, the Department of Defense, or the US Government.

Presented at the meeting of the Virginia Society of Otolaryngology, Reston, Virginia, May 7-8, 1999.

CORRESPONDENCE — John H. Greinwald, Jr, MD, Dept of Otolaryngology, Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH 45229.

DATA ON PATIENTS WITH ENDOBRONCHIAL TUMORS

Age	*Sex*	*Race*	*Location of Tumor*	*Signs/Symptoms*	*Interventions*	*Diagnosis*
9 y	F	White	RMB	Pneumonia	Segmental resection	Mucoepidermoid carcinoma
2 y	F	White	LMB	Wheezing	Endoscopic resection	Hemangioma
11 mo	M	White	LMB	Wheezing	Endoscopic resection (argon laser)	Epithelioid granuloma (histoplasmosis)
6 y	M	White	Bilateral	SOB, cough, hoarseness	Endoscopic resection	Tracheobronchial papilloma
12 y	F	White	RMB	Pneumonia	Segmental resection	Bronchial carcinoid
13 y	M	White	LMB	Bronchitis, wheezing	Endoscopic resection	Epithelioid granuloma (histoplasmosis)
12 y	M	White	Bilateral	SOB, cough, hoarseness	Endoscopic resection	Tracheobronchial papilloma
2 y	F	African American	RMB	Cough, pneumonia, HIV	Endoscopic resection	Leiomyoma
4 y	M	Hispanic	Bilateral	SOB, wheezing	Endoscopic resection	Tracheobronchial papilloma
23 mo	M	White	RMB	Wheezing	Endoscopic resection	Epithelioid granuloma (histoplasmosis)

RMB — right main stem bronchus, LMB — left main stem bronchus, SOB — shortness of breath, HIV — human immunodeficiency virus.

Adjuvant therapy with interferon, indole-3-carbinol, or methotrexate also was used to stabilize disease progression in 2 patients; the third patient died of the disease.

Inflammatory masses of the main bronchi were diagnosed in 3 children and were submitted to biopsy and resected; in 1 of these cases, an argon laser was used to complete the removal of the mass. Pathological examination showed noncaseating histiocytic granulomas clinically consistent with histoplasmosis. Two of these lesions responded to surgical treatment alone, and 1 child underwent adjuvant itraconazole therapy for tumor resolution.

An endobronchial hemangioma, in conjunction with a large cervical hemangioma, was treated with corticosteroid therapy. One patient underwent successful endobronchial resection of a leiomyoma. Four case reports highlight the diagnostic and therapeutic challenges of these masses.

Case 1. A 2-year-old, previously healthy boy was referred for pulmonary evaluation after a 3-month history of recurrent bronchitis and wheezing; no improvement was noted with antibiotic or bronchodilator therapy. There was no prior history of fevers, night sweats, chronic cough, immunocompromise, or foreign body aspiration. His family lived on a farm in the rural Midwest. A chest radiograph revealed a radiodense lesion in the upper right hilum. A tuberculin skin test was negative. Flexible fiberoptic bronchoscopy was performed, revealing a mass of the

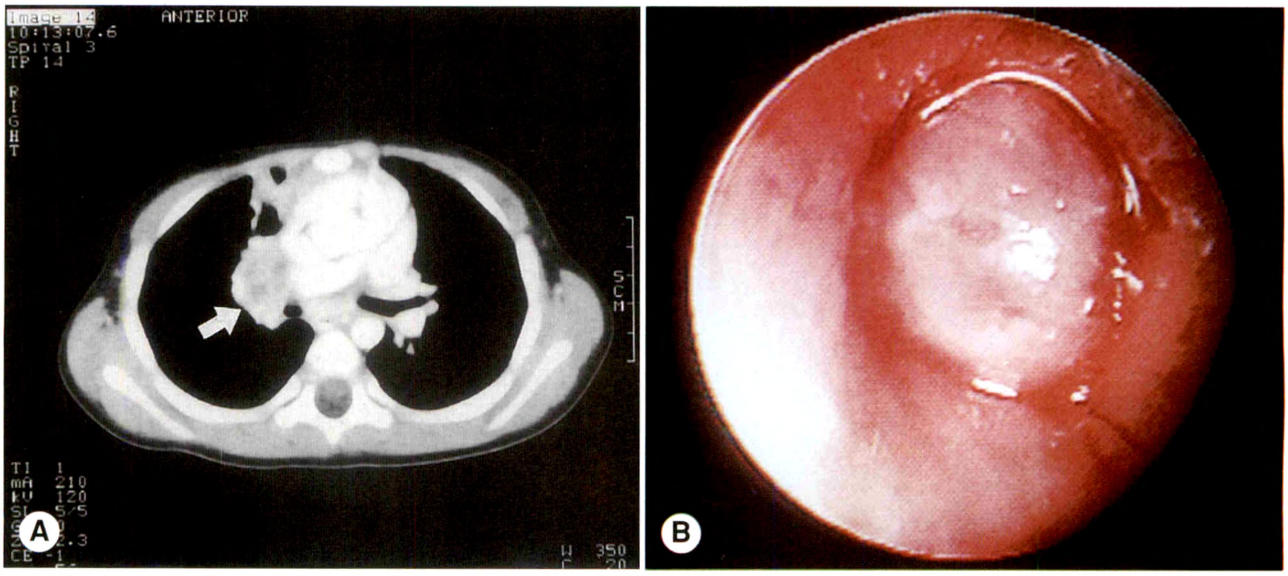


Fig 1. (Case 1) A) Computed tomography scan of chest demonstrates right mediastinal mass abutting right main stem bronchus (arrow). Note multiple low-attenuation areas within mass. B) Endoscopic photograph demonstrates soft, pink, polypoid mass completely obstructing right bronchus intermedius. Mild proximal mucosal edema is present.

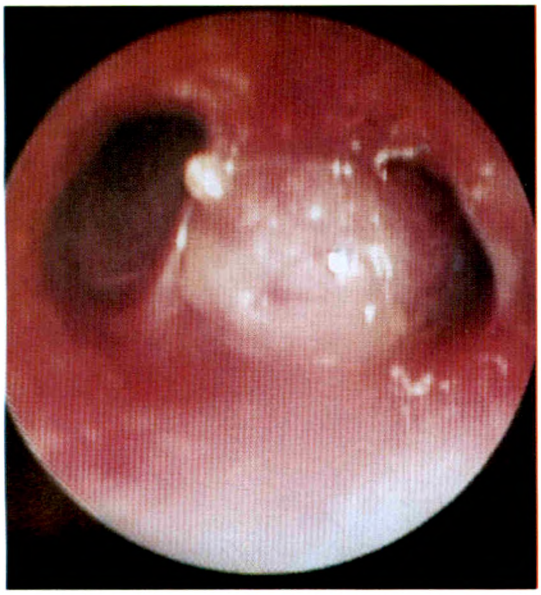


Fig 2. (Case 2) Endoscopic photograph of carina shows pale, fleshy, pedunculated mass arising from right main stem bronchus and obstructing more than half of lumen.

right main stem bronchus. Subsequent CT examination of the chest (Fig 1A) confirmed the findings of plain radiography, and the patient was referred for a biopsy of the mass.

At rigid bronchoscopy, a grayish, polypoid mass was found filling the right main bronchus (Fig 1B). The mass was friable, but did not bleed easily. With a combination of optical cup forceps and an argon laser (300-nm cable, 3 W, 0.5-second duration), the mass was debrided in its entirety from the airway wall, revealing evidence of erosion. The pathological findings were remarkable for noncaseating epitheloid granulomas with numerous histiocytes. Special stains for fungi and acid-fast bacilli were negative, and all cultures also were negative.

The clinical presentation, in combination with the radiographic and pathological findings, led to a presumptive diagnosis of blastomycosis or histoplasmosis. Histoplasma serologies revealed a negative IgG and a weakly positive IgM, suggesting recent infection. Blastomyces serologies were negative for IgM and weakly positive for IgG. A repeat CT scan performed 3 months later showed resolution of the mass, and clinical evaluation demonstrated no residual symptoms.

Case 2. A 9-year-old girl had presented with a history of recurrent right pneumonia 4 times over the past year; each episode was treated successfully with antibiotics. She was otherwise healthy, with no growth retardation or other infectious disorders (ie, otitis, sinusitis). Plain chest radiographs demonstrated a persistent right lower lobe infiltrate. A CT evaluation of the chest revealed subcarinal adenopathy and a right lower lobe infiltrate.

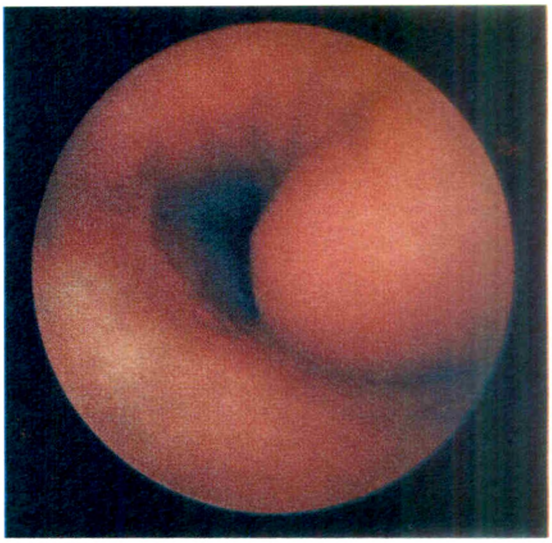


Fig 3. (Case 3) Endoscopic photograph shows slightly erythematous, smooth, compressible lesion in left main stem bronchus.

The otolaryngology service was consulted. By physical examination, rhonchi could be heard in the right lung fields, and on rigid bronchoscopy, a fleshy, exophytic, pedunculated 1.5-cm mass was found in the proximal right bronchus intermedius, extending to the opening of the right main bronchus (Fig 2). Cultures obtained for aerobic and anaerobic bacteria, fungi, and tuberculosis were negative. A biopsy specimen was obtained endoscopically, and pathological examination showed sheets of polygonal squamous epithelial cells with large pleomorphic vesicular nuclei showing prominent nucleoli consistent with an epidermoid tumor. A significant component of mucus-secreting cells also was present, and a diagnosis of mucoepidermoid carcinoma was made.

The pediatric surgical service was consulted, and the patient underwent an uncomplicated en bloc resection of the right middle and lower lobes. Mediastinal lymph node dissection demonstrated numerous small, nonpathological nodes. The final pathological diagnosis was low-grade mucoepidermoid carcinoma with clear surgical margins. Radiotherapy was not used, and the patient has been without evidence of disease for more than 6 years.

Case 3. A 2-year-old girl was seen by the otolaryngology service with a slowly enlarging right level 4 neck mass that had been present for more than 6 months. The mass was soft and compressible, with no overlying skin changes. The child also had a history of wheezing and recurrent bronchitis; there was no history of fever, chills, weight loss, or change in appetite.

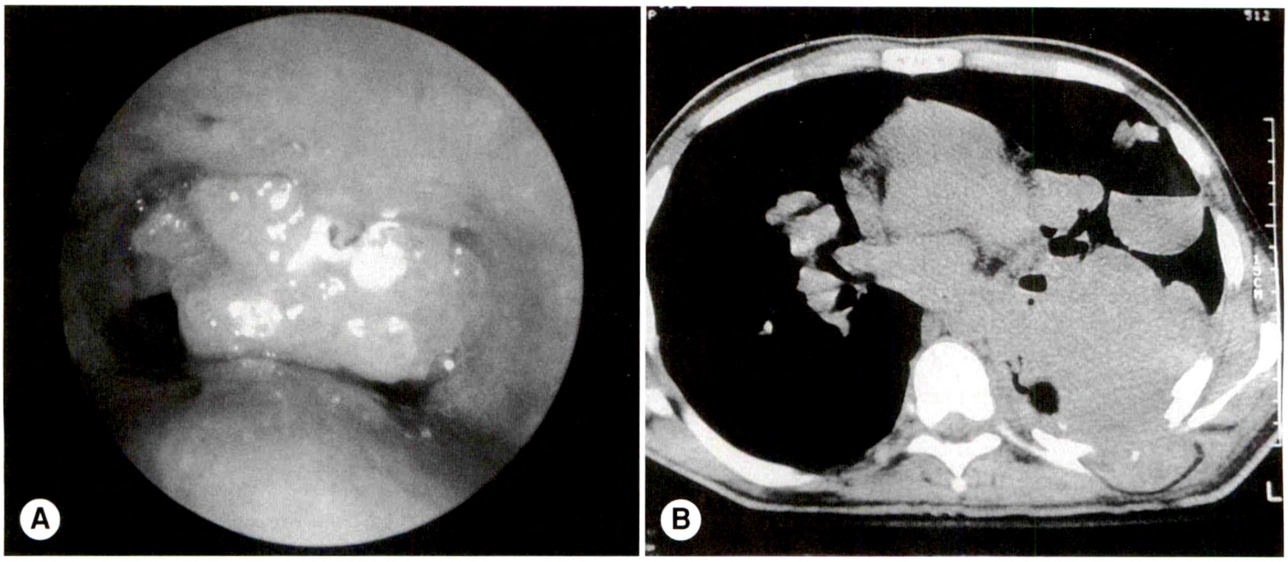


Fig 4. (Case 4) A) Diffuse, obstructing tracheobronchial papillomas are seen on endoscopic photography during bronchoscopy. B) Computed tomography scan of chest demonstrates significant soft tissue infiltration of pulmonary parenchyma by papilloma.

The CT scans revealed a lobulated right 3 × 4-cm lower cervical mass that enhanced with contrast. In addition, a small mass with similar characteristics also was identified in the left anterior mediastinum, adjacent to the left main stem bronchus. Open cervical biopsy revealed a lobulated, hyperemic mass consistent with hemangioma. Bronchoscopy demonstrated a soft, compressible, mucosa-covered mass on the medial wall of the left main bronchus (Fig 3), which was diagnosed as an endobronchial hemangioma. Postobstructive mucus retention was treated with bronchoalveolar lavage. Aerobic and anaerobic cultures were negative for growth.

The patient underwent oral corticosteroid therapy (2 mg/kg) for 8 weeks. Aggressive pulmonary toilet and numerous courses of antibiotics were used over many months. Slow resolution of the cervical mass was noted over the next 12 months, with complete resolution documented more than 2 years from the time of diagnosis. The endobronchial lesion similarly resolved over a 3-year period, compatible with the natural involutional process of hemangiomas.

Case 4. An 18-year-old boy had initially presented to the otolaryngology service at 2 years of age with hoarseness. Direct laryngoscopy confirmed laryngeal papillomatosis, and a CO_2 laser was used to excise the disease. He briefly required a tracheotomy and underwent numerous procedures to resect and vaporize the disease. Tracheal papillomas with bronchial invasion were diagnosed at 6 years of age (Fig 4A), and a CT scan of the chest confirmed pulmonary parenchymal involvement. He was given 3 million U/m^2 of interferon α-2a and has remained on this medication.

The patient's pulmonary disease continued to progress, despite repeated bronchoscopic resections and additional adjunctive therapy with acyclovir and indole-3-carbinol (Fig 4B). Resection of papillomas has been made appreciably more precise with the use of laryngeal microdebriders; minor bleeding is controlled with oxymetazoline hydrochloride–soaked pledgets. In consultation with the hematology service, methotrexate sodium (37.5 mg by mouth weekly) was prescribed when the patient was 16 years of age (1996); the bronchopulmonary disease stabilized, as judged by serial CT examinations of the chest. The patient remains active and in school.

DISCUSSION

Endobronchial tumors are uncommon in the pediatric population, with fewer than 200 cases in the English-language literature. Hancock et al[2] summarized 383 cases in the literature of pulmonary parenchymal tumors and primary endobronchial masses presenting in childhood, including 234 cases previously reviewed by Hartman and Shochat.[1] Although there were many different histologic diagnoses, more than 70% of the endobronchial tumors they reported were either bronchial adenomas (which included carcinoid and mucoepidermoid tumors) or bronchogenic carcinomas. Relatively little attention has been given to the diagnosis and treatment of rarer endobronchial tumors, particularly those of a benign origin. Recent advances in surgical and adjuvant therapies for these tumors have enhanced our ability to treat them. As experienced bronchoscopists, otolaryngologists are often the first clinicians to evaluate these lesions, and therefore a familiarity with all aspects of the pathophysiology and treatment of endobronchial tumors is warranted.

Most of the tumors in our series presented in preschool or school-aged children who had relatively nonspecific respiratory complaints (ie, cough and wheezing). No gender or location bias was demonstrable, consistent with the report by Hartman and Shochat.[1] However, we did note an ethnic bias that most likely represents an ascertainment artifact. The fact that all of our patients with chronic cough and wheezing were treated for prolonged periods of time for common conditions, especially asthma, suggests that if an appropriate response to medical therapy is not forthcoming, a more thorough examination of the airway should be done. As Jackson[4] admonished, "All is not asthma that wheezes."

The basic examination should include anterior-posterior and lateral chest radiographs, which may demonstrate a persistent unilateral infiltrate or frank pneumonia, as demonstrated by cases 1 and 2. Computed tomography also is helpful in assessing the extent of the primary lesion and determining the presence of mediastinal lymph nodes, bony erosion, or pulmonary parenchymal involvement.[5] Bronchoscopy, with biopsy and lavage for culture and cytologic evaluation, is required for a definitive diagnosis.[2,5,6] In cases 1 through 4, both CT and bronchoscopy were essential for proper diagnosis. Therapeutic resection of the obstructing endobronchial lesion provides definitive therapy, improves pulmonary function, and obviates open surgical procedures. Other tests, such as barium esophagrams or swallow studies, pulmonary function tests, and angiography, may be performed as dictated by the patient's presentation, but often, these tests are not required.

Histoplasmosis is a common fungal infection of adults and children caused by *Histoplasma capsulatum*. It is endemic in the Mississippi River and Ohio River valleys, where 50% to 100% of the population have positive skin tests.[7,8] On initial exposure, immunocompetent persons develop flulike symptoms that do not warrant medical attention. Although chest radiographs may appear normal, pulmonary nodules, infiltrates, and enlarged mediastinal lymph nodes are not unusual.[9] For example, Lerner and DeLuca[10] described a patient in whom enlarged mediastinal lymph nodes caused bronchial obstruction and a persistent cough. Because *H capsulatum* is only rarely recovered by culture or staining techniques,[11] adjuvant diagnostic techniques, such as complement fixation titers, should be used if histoplasmosis is highly suspected. The CT findings that are consistent with this diagnosis are the presence of mediastinal lymph nodes with low attenuation and enhancing septae (Fig 1A).[12] All 3 children with histoplasmosis in this series had this finding, although serologies were positive in only 2.

To our knowledge, obstructive endobronchial granulomatous tissue extending from mediastinal lymph nodes has not been previously reported, although other investigators have noted extrinsic compression and nonobstructive ulceration of the bronchi due to enlarged granulomatous mediastinal lymph nodes.[12,13] The patients we treated presented with soft, pale red masses mimicking endobronchial granulation tissue (Fig 1B). The primary consideration in the differential diagnosis is inflammatory pseudotumors — idiopathic masses that are grossly and histologically quite distinct from histoplasmosis. In contrast to the caseating and noncaseating granulomatous inflammation of histoplasmosis, inflammatory pseudotumors are composed of plasma cells with various amounts of granulation tissue, lymphocytes, and large mononuclear cells.[14] Other entities that should be considered are tuberculosis, toxoplasmosis, Langerhans' cell histiocytosis, and chronic foreign body reactions.

In most persons with localized pulmonary and/or mediastinal histoplasmosis, spontaneous resolution occurs, making treatment unnecessary.[9,15] The 3 children we treated underwent endoscopic resection of the obstructing granulomatous tissue, in 1 case with the assistance of an argon laser. Two patients responded without further treatment, but in 1, adjuvant oral itraconazole was given empirically on the recommendation of a consultant. Itraconazole (3 to 5 mg/kg per day) given for 2 to 3 months has been proposed for children with symptomatic disease, although there is only limited experience to justify its routine use.[16] We would recommend reserving systemic antifungal therapy (ie, amphotericin B or itraconazole) for severe, progressive, recurrent, or life-threatening *H capsulatum* infection.[17]

Hemangiomas are the most common tumor of infancy. Although they often manifest as innocuous red, macular lesions that involute by 5 to 7 years of age,[18] in about 5% of children, cosmetic or life-threatening complications necessitate treatment (ie, airway compromise, thrombocytopenia).[19] Lesions of the subglottis and cervical trachea are not uncommon, and their presentation and treatment have been well described.[20,21] However, distal tracheal and endobronchial hemangiomas are rare. Woolley and Lusk[22] reported a nonobstructing lesion of the distal trachea in a newborn who also had a 50% obstructing, noncontiguous subglottic lesion. The tracheal lesion was observed, and no long-term follow-up data were given. Cohen and Kaschula[23] described a child with an endobronchial hemangioma that caused a cough, bronchitis, and pneumonia. The lesion was excised by wedge resection of a portion of the right lung. Unfortunately, in this case also, few details are given. Other authors have reported on intraparenchymal pulmonary hemangiomatosis[24,25] and vascular "angiomatous" tracheal lesions in adults[26]; however, to the best of our knowledge, our case represents only the second report of a pediatric endobronchial hemangioma.

The patient we treated (case 3) presented with a pink, soft, mucosa-covered, compressible mass (Fig 3). The diagnosis was confirmed by CT, along with that of a similar-appearing neck mass that was a biopsy-proven cervical hemangioma. A short trial of corticosteroid therapy was used, but it is difficult to say whether it had any effect. If this child presented today, other treatment options also would be considered. For example, interferon therapy has come to the forefront of treatment for significant hemangiomas. Greinwald et al[27] reported on a series of 24 patients with massive or potentially life-threatening hemangiomas; 60% responded to interferon therapy, a response rate that is generally better than that seen with corticosteroid therapy as reported by Enjroles et al.[28] Endoscopic or open resection also could be considered, if medical therapy is ineffective or complete obstruction mandates aggressive intervention.[6] The former requires cable-delivered lasers, such as Nd:YAG or green light lasers (argon or KTP).[29]

Like hemangiomas, laryngeal papillomas are common, but again, tracheobronchial involvement is rare.[6,30-32] Our patients all presented in the first decade of life with a significant history of laryngeal disease. All had required tracheotomy at some time, and although decannulation was eventually possible, each child required numerous procedures to treat tracheal disease with forceps, CO_2 laser vaporization, or laryngeal microdebriders. Adjuvant therapy, including interferon, acyclovir, and indole-3-carbinol, was used in all 3 patients, with 1 of the 3 achieving disease stabilization.[30,33] The 2 remaining patients required weekly methotrexate to stabilize the progression of tracheobronchial and pulmonary papilloma growth. One patient died of the disease. Recently, cidofovir has been found to be of great benefit in laryngeal papillomas.[34] Future studies will be required to confirm these preliminary results in the larynx and to establish whether cidofovir may be useful in the treatment of tracheal papillomas.

Leiomyomas are benign tumors of smooth muscle. Endobronchial leiomyomas have been reported previously in only 1 pediatric patient.[35] This child presented with unilateral wheezing and bronchitis, similar to the patient in our series. The HIV infection in our patient was most likely coincidental. The other patient required lobectomy for adequate treatment. With the advent of fiberoptic technology, benign sessile lesions of the bronchus can be adequately treated endobronchially. The patient in our series underwent successful resection with cup forceps. Now, we would recommend an Nd:YAG or KTP (or argon) laser to assist in hemostasis.

We also treated 2 children with malignant tumors that presented as endobronchial masses. The primary role of the otolaryngologist in the care of these patients is as the diagnostician, because intrathoracic resection is required for complete tumor extirpation. Malignant bronchial tumors typically present like benign lesions, causing cough, wheezing, and bronchitis. In addition, hemoptysis is seen in one third of children with malignant lesions.[2] The diagnosis of suspected malignant endobronchial tumors should be made on pathological examination after rigid bronchoscopic biopsy.

Bronchial adenomas or carcinoid tumors, together with inflammatory lesions of the bronchus, are the most common endobronchial tumors in children, each accounting for more than 12% of cases.[1] Although pathologically benign-appearing on histologic examination, adenomas present with regional and distant metastasis in 5% to 10% of patients.[3] For this reason, a staging CT examination of the chest should be performed. In children, even in the presence of local invasion and high-grade tumors, segmental resection and lobectomy offers a 90% survival rate.[1]

Mucoepidermoid tumors are the second most common malignant bronchial tumors, although in some series they are included as "bronchial adenomas."[36] Their presentation and diagnostic evaluation are similar to those of carcinoid tumors.[37] Usually of a low histologic grade, they rarely show invasion or metastasis. Treatment consists of segmental bronchial resection with or without adjacent segmental lobectomy. Adjuvant irradiation or chemotherapy is indicated only rarely,[38] because the survival rate of pediatric patients is greater than 90% with surgery alone.[1,38] The patient in our series has no evidence of recurrence more than 5 years after his surgical resection without the use of adjuvant therapy.

SUMMARY

Endobronchial tumors in children are rare, with fewer than 200 cases reported in the English-language literature. The diagnosis of this heterogeneous group of tumors requires a high index of suspicion in children with atypical or chronic respiratory complaints. Although the prognosis in the majority of children with endobronchial tumors is excellent, malignant tumors require aggressive treatment, and so-called benign disorders like papillomas can progress to distal tracheobronchial and pulmonary involvement, which often portends a high rate of morbidity and possible mortality.

REFERENCES

1. Hartman G, Shochat SJ. Primary pulmonary neoplasms of childhood: a review. Ann Thorac Surg 1983;36:108-19.

2. Hancock BJ, DiLorenzo M, Youssef S, Yazbeck S, Marcotte J, Collin P. Childhood primary pulmonary neoplasms. J Pediatr Surg 1993;28:1133-6.

3. Lack E, Harris G, Eraraklis A, Vawter G. Primary bronchial tumors in childhood. Cancer 1983;51:492-7.

4. Jackson C. Wheezing respiration in children. Am J Dis Child 1931;41:153-7.

5. Tischer W, Reddemann H, Herzog P, et al. Experience in surgical treatment of pulmonary and bronchial tumours in childhood. Prog Pediatr Surg 1987;21:118-35.

6. Desai DP, Holinger LD, Gonzalez-Crussi F. Tracheal neoplasms in children. Ann Otol Rhinol Laryngol 1998;107:790-6.

7. Hasleton PS. Pulmonary mycotic disease. In: Hasleton PS, ed. Spencer's pathology of the lung. New York, NY: McGraw-Hill, 1996:273-301.

8. Chintapalli KN, Gurney JW, Glenn ME, Sharkey PK. Mycotic pulmonary infections. Crit Rev Diagn Imaging 1992;33:231-92.

9. Maxson S, Jacobs RF. Community-acquired fungal pneumonia in children. Semin Respir Infect 1996;11:196-203.

10. Lerner MH, DeLuca SA. Mediastinal granuloma secondary to histoplasmosis. Am Fam Physician 1991;43:1649-51.

11. Blumenfeld W, Gan LG. Diagnosis of histoplasmosis in bronchoalveolar lavage fluid by intracytoplasmic localization of silver-positive yeast. Acta Cytol 1991;35:710-2.

12. Landay MJ, Rollins NK. Mediastinal histoplasmosis granuloma: evaluation with CT. Radiology 1989;172:657-9.

13. Kneale B, Turton C. Bronchoscopic findings in a case of bronchopulmonary histoplasmosis. Thorax 1995;50:314-5.

14. Bahadori M, Liebow AA. Plasma cell granulomas of the lung. Cancer 1973;31:191-208.

15. Sarosi GA. Community-acquired fungal diseases. Clin Chest Med 1992;12:337-47.

16. Bullock WE. Principles and practice of infectious disease. New York, NY: Churchill Livingstone, 1995:2340-53.

17. Johnson P, Sarosi G. Current therapy of major fungal diseases of the lung. Infect Dis Clin North Am 1991;5:635-45.

18. Ezekowitz RA, Mulliken JB, Folkman J. Interferon alpha-2a therapy for life threatening hemangiomas of infancy. N Engl J Med 1992;326:1456-63.

19. Weber TR, Connors RH, Tracy TF, Bailey PV. Complex hemangiomas of infants and children. Arch Surg 1990;125:1017-21.

20. Ohlms LA, Jones DT, McGill TJI, Healy GB. Interferon alfa-2a therapy for airway hemangiomas. Ann Otol Rhinol Laryngol 1994;103:1-8.

21. Bartlett JA, Riding KH, Salkeld LJ. Management of hemangiomas of the head and neck in children. J Otolaryngol 1988;17:111-20.

22. Woolley AL, Lusk RP. Multiple vascular lesions of an infant's airway. Otolaryngol Head Neck Surg 1994;111:305-8.

23. Cohen MC, Kaschula RO. Primary pulmonary tumors in childhood: a review of 31 years' experience and the literature. Pediatr Pulmonol 1992;14:222-32.

24. Whittaker JS, Pickering CAC, Heath D, Smith P. Pulmonary capillary hemangiomatosis. Diagn Histopathol 1983;6:77-84.

25. Katzenstein AL, Gmelich JT, Carrington CB. Sclerosing hemangioma of the lung. Am J Surg Pathol 1980;4:343-56.

26. Cavaliere S, Foccoli P, Farina PL. Nd:YAG laser bronchoscopy: a five-year experience with 1,396 applications in 1,000 patients. Chest 1988;94:15-21.

27. Greinwald J, Burke D, Bonthius D, Bauman NM, Smith RJH. An update on the treatment of hemangiomas in children with interferon alfa-2a. Arch Otolaryngol Head Neck Surg 1999;125:21-7.

28. Enjroles O, Riche MC, Merland JJ, Escande JP. Management of alarming hemangiomas in infancy: a review of 25 cases. Pediatrics 1990;85:491-8.

29. Rimell FL. Pediatric fiberoptic laser rigid bronchoscopy. Otolaryngol Head Neck Surg 1996;114:413-7.

30. Bauman N, Smith R. Recurrent respiratory papillomatosis. Pediatr Clin North Am 1996;43:1385-401.

31. Cohen SR, Geller KA, Seltzer S, Thompson JW. Papilloma of the larynx and tracheobronchial tree in children: a retrospective study. Ann Otol Rhinol Laryngol 1980;89:497-503.

32. Solomon D, Smith RRL, Kashima H, Leventhal BG. Malignant transformation in non-irradiated recurrent respiratory papillomatosis. Laryngoscope 1985;95:900-4.

33. Derkay CS. Task force on recurrent respiratory papillomas: a preliminary report. Arch Otolaryngol Head Neck Surg 1995;121:1386-91.

34. Pransky SM, Magit AE, Kearns DB, Kang DR, Duncan NO. Intralesional cidofovir for recurrent respiratory papillomatosis in children. Arch Otolaryngol Head Neck Surg 1999;125:1143-8.

35. Sweet RS. Pulmonary leiomyoma. Am J Roentgenol Radium Ther Nucl Med 1969;107:823-6.

36. Mullins J, Barnes R. Childhood bronchial mucoepidermoid tumors: a case report and review of the literature. Cancer 1979;44:315-22.

37. Seo S, Warren J, Mirkin L, Weisman SJ, Grossfield JL. Mucoepidermoid carcinoma of the bronchus in a 4-year-old child, a high-grade variant with lymph node metastasis. Cancer 1984;53:1600-4.

38. McDougall JC, Gorenstein A, Unni K, O'Connell EJ. Carcinoid and mucoepidermoid carcinoma of bronchus in children. Ann Otol Rhinol Laryngol 1980;89:425-7.

Ann Otol Rhinol Laryngol 110:2001

CONGENITAL NASAL PYRIFORM APERTURE STENOSIS: DIAGNOSIS AND MANAGEMENT OF 20 CASES

THIERRY VAN DEN ABBEELE, MD, PHD
PARIS, FRANCE
MARTINE FRANÇOIS, MD
PARIS, FRANCE

JEAN-MICHEL TRIGLIA, MD
MARSEILLE, FRANCE
PHILIPPE NARCY, MD
PARIS, FRANCE

The objective of this study was to review the characteristics of congenital nasal pyriform aperture stenosis (CNPAS) in a series of 20 children seen between 1993 and 1996. The diagnosis was made by physical examination and computed tomography scan. A single central maxillary incisor was detected in 12 cases (60%). Three children had morphological abnormalities of the pituitary gland shown on magnetic resonance imaging. One child had an antidiuretic hormone deficiency, and another child had a growth hormone deficiency. Two children had craniosynostoses, 1 of which was Apert's syndrome. All patients underwent operation by a sublabial approach, and 1 was referred for a columellar necrosis after nasal stenting. After surgery, all patients showed improvement, and the nasal stenting was usually removed 1 week after surgery. Follow-up revealed normal breathing. In conclusion, CNPAS was previously considered to be an unusual cause of nasal obstruction in neonates and infants. The number of cases treated recently in our department suggests that this newly recognized entity is more common than expected.

KEY WORDS — infant, nasal obstruction, neonate, pyriform aperture stenosis.

INTRODUCTION

Congenital nasal pyriform aperture stenosis (CNPAS) has been described as a new and rare disorder that causes airway obstruction in newborns and infants. The first cases were reported by Brown et al[1] in 1989, and no more than 20 cases have been published since then. The association of CNPAS with midfacial and midbrain abnormalities has been recently underlined,[2-4] and a relationship with holoprosencephaly has been reinforced by the occasional finding of chromosomal abnormalities.[5] This report describes 20 new cases of CNPAS managed in our department from 1993 to 1996. This number suggests that the incidence of CNPAS has been underestimated. Nineteen of the 20 children were neonates or infants and were successfully managed by a surgical enlargement of the pyriform aperture followed by a short period of nasal stenting. The last child presented with a chronic nasal obstruction and a septocolumellar necrosis caused by nasal stents during the neonatal period and was operated on at the age of 4 years. The diagnosis was suspected on the basis of clinical features, and a 3.5-mm nasofiberscope could not pass through the pyriform aperture in any of the children. A computed tomography (CT) scan confirmed the diagnosis in all cases. The clinical features, relationships with craniofacial and encephalic abnormalities, and management are discussed.

PATIENTS AND METHODS

Twenty children (10 boys and 10 girls) 1 week to 6 months of age were managed between 1993 and 1996. The average follow-up (±SD) was 32 ± 11 months. All surgical procedures were performed identically, with enlargement of the pyriform aperture and postoperative nasal stenting by endotracheal tubes. Briefly, a sublabial approach was used to expose the pyriform aperture, the nasal mucosa was gently dissected, and the external bony margins were drilled out with small diamond burs. The lacrimonasal ducts were often exposed, but never injured. When present, the nasal spine was always respected. Endotracheal tubes were then secured in place with a loose transcolumellar suture, and their size was adapted to the age and weight of the children. The airway patency was assessed after operation with a 3.5-mm flexible endoscope (Olympus ENF P3) in all cases. The Table summarizes the clinical course for each patient. All patients presented with nasal obstruction, and half of them with immediate neonatal respiratory distress. The delay between the beginning of the medical therapy and the surgical procedure ranged from 8 days to 1 year (mean, 74 days). The 6 illustrative cases described below provide more precise information

---

From the Department of Pediatric Otorhinolaryngology, Robert Debré Hospital, Paris (Van Den Abbeele, François, Narcy), and the Department of Otorhinolaryngology, La Timone Hospital, Marseille (Triglia), France.

Presented at the meeting of the American Society of Pediatric Otorhinolaryngology, Orlando, Florida, May 6-9, 1996.

CORRESPONDENCE — Thierry Van Den Abbeele, MD, PhD, Service d'ORL pédiatrique, Hôpital Robert Debré, 48 Boulevard Sérurier, 75019 Paris, France.

CASES OF CONGENITAL NASAL PYRIFORM APERTURE STENOSIS

Case No.	*Sex*	*Symptoms*	*Onset (d)*	*Nose*	*Medial Upper Incisor*	*Genetic History*	*MRI Findings*	*Delay of Surgery (d)*	*Stenting Duration (d)*	*Follow-up (mo)*	*Nasal Airway*
1	M	Distress	0	Narrow, flat bridge, hypotelorism, septocolumellar necrosis	Yes		Normal	4 y	5 mo (neonatal) 21 d (repair)		Subnormal (septal perforation)
2*	F	Distress	11	Narrow	Yes			8	28	54	Normal
3	M	Obstruction	30	Narrow, flat bridge, Apert's syndrome	Yes	Normal karyotype		311	14	41	Normal
4*	F	Distress	0	Narrow, flat bridge, hypotelorism	No	Normal karyotype, consanguinity	Chiari I, hypophyseal agenesis	15	7	48	Normal
5	M	Distress	0	Narrow	Yes	Normal karyotype, medial incisor in brother		323	10	32	Subnormal (septal deviation)
6	M	Obstruction	10	Narrow, flat bridge, hypotelorism	No	Normal karyotype, spontaneous abortions	Hypoplastic corpus callosum	60	7	34	Subnormal (granulations)
7*	F	Distress	0	Narrow, flat bridge, hypotelorism	Yes	Normal karyotype	Hypophyseal ectopy	20	9	35	Normal
8	F	Distress	0	Narrow	No			45	5	33	Normal
9*	M	Distress	0	Narrow	No			9	5	28	Normal
10	M	Obstruction	0	Narrow	Yes	Bilateral cleft in father		82	7	24	Normal
11*	F	Obstruction	0	Narrow, flat bridge, hypotelorism	Yes			11	2		Restenosis
								30	7	22	Normal
12*	M	Obstruction	0	Narrow	Yes			9	2		Partial restenosis
									10	22	Normal
13	M	Obstruction	5	Narrow	No			36	2	17	Normal
14*	M	Distress	0	Narrow	No			8	21	18	Normal
15	F	Obstruction	3	Narrow, craniosynostosis	Yes	Normal karyotype	Normal	40	6	16	Normal
16	M	Obstruction	0	Narrow	No			36	6	15	Normal
17	F	Distress	0	Narrow	Yes		Normal	60	22	12	Normal
18	F	Distress	0	Narrow	Yes		Normal	137	7	6	Normal
19	F	Obstruction	0	Narrow, flat bridge, hypotelorism	No	Normal karyotype	Normal	45	10	9	Subnormal (granulations)
20	F	Obstruction	0	Narrow, flat bridge, hypotelorism	Yes	Normal karyotype, consanguinity	Hypophyseal agenesis	89	9	7	Normal
Mean								70.7	9.4	24.9	
SD								93.1	7.1	13.7	

MRI — magnetic resonance imaging.

*Patients operated on within 30 days of onset of symptoms.

and are selected for discussion of relationships with other midfacial or midbrain abnormalities. (Case numbers refer to the Table.)

Case 1. A male infant, the product of a normal 40-week pregnancy, showed immediate obstructive respiratory distress. A CT scan eliminated bilateral pos-

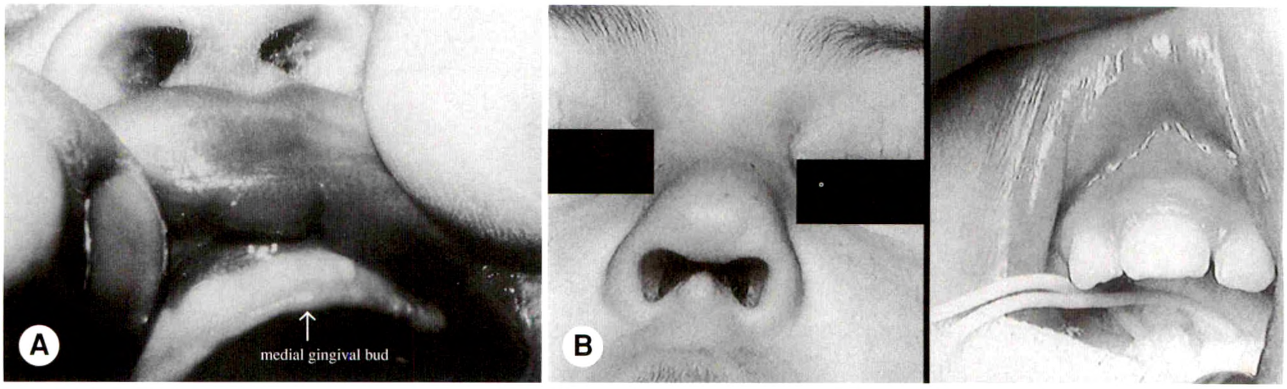


Fig 1. Clinical features of congenital nasal pyriform aperture stenosis (CNPAS). A) (Case 5) Diagnosis of single upper central incisor in infant before teething. Note absence of upper gingival frenulum and intermaxillary suture. Note medial gingival bud and narrowing of nostril apertures. B) (Case 1) CNPAS associated with midline abnormalities in 4-year-old child. Left image shows necrosis of columella caused by prolonged stenting. Right image shows central megaincisor. Note absence of upper labial frenulum and intermaxillary suture.

terior choanal atresia. The child was treated with bilateral prolonged nasal stenting (4-mm nasal stents for 5 months). He was then referred at 6 months of age to our department for septocolumellar necrosis, obviously caused by the prolonged stenting (Fig 1B). Clinical examination revealed a single medial upper gingival bud and necrosis of the anterior part of the cartilaginous septum and the columella. Reviewing the previous CT scan showed a typical central megaincisor and bony stenosis of the pyriform apertures. The nasal stents were removed, and the nasal airway was found to be large enough for conservative treatment. A septocolumellar reconstruction with an auricular cartilage graft and enlargement of the pyriform apertures was successfully performed at the age of 4 years, but required 3 staged surgical procedures and nasal stenting for 3 weeks.

Case 3. A male infant, the product of a normal gestation, presented with a craniosynostosis and bilateral syndactyly forming a typical Apert's syndrome and gradually developed an obstructive neonatal rhinitis attributed to his facial dysmorphy. The craniosynostosis was operated on at 2 months, but the nasal obstruction remained unchanged after 8 months. Clinical examination revealed a selective narrowing of the anterior part of the nasal airway. A CT scan revealed CNPAS and a medial upper incisor bud. His brain and pituitary gland were normal. A sublabial approach was used, and bilateral nasal stenting with 3-mm endotracheal tubes was secured in place for 14 days. Nasal ventilation was greatly improved after removal of the stents and remains stable after a 1-year follow-up. An upper central megaincisor has appeared since then.

Case 4. A female infant, the product of a 39-week normal pregnancy, had parents who were first cousins and had previously had a normal female child. Her birth weight was 2,830 g, her length 47 cm, and her head circumference 32 cm. She presented with immediate respiratory distress that was relieved by crying and an oral cannula. She was treated for 10 days with an oral cannula and fed by gavage, and was then referred to our department. It was impossible to catheterize the nasal airway with small probes, and topical epinephrine drops were ineffective. A CT scan and clinical examination showed a narrowing of the pyriform aperture and a large nasal bridge, but no upper central incisor. The nasal obstruction was greatly improved after the surgery, and the nasal stenting was removed after 1 week. Her postoperative breathing was improved, but she had severe episodes of hypoglycemia, and septicemia with *Staphylococcus aureus*–positive hemocultures. During the subsequent follow-up, she was found to be suffering from panhypopituitarism with growth hormone, thyroid hormone, and cortisol deficiencies. A magnetic resonance imaging (MRI) examination revealed total pituitary agenesis (Fig 2A) and a Chiari type I malformation. The corpus callosum was normal. After a 6-month follow-up, she was found to be developmentally retarded. Her karyotype was normal.

Case 5. A 6-month-old male infant, the product of a normal pregnancy, presented several hours after birth with immediate obstructive respiratory distress, was treated with local and general corticosteroids (0.2 mg/kg per day of oral betamethasone sodium phosphate) for several weeks. He was given a 7-week nasal stenting, because each episode of viral rhinitis was marked by sleep apnea and topical epinephrine drops were poorly effective. As a consequence of this prolonged corticotherapy, he developed a coraliform renal lithiasis that required extracorporeal lithotripsy. At 6 months, clinical examination revealed an anterior narrowing of the pyriform apertures, and a flexible nasopharyngoscope could not be passed through the left nostril. Mouth examination revealed the ab-

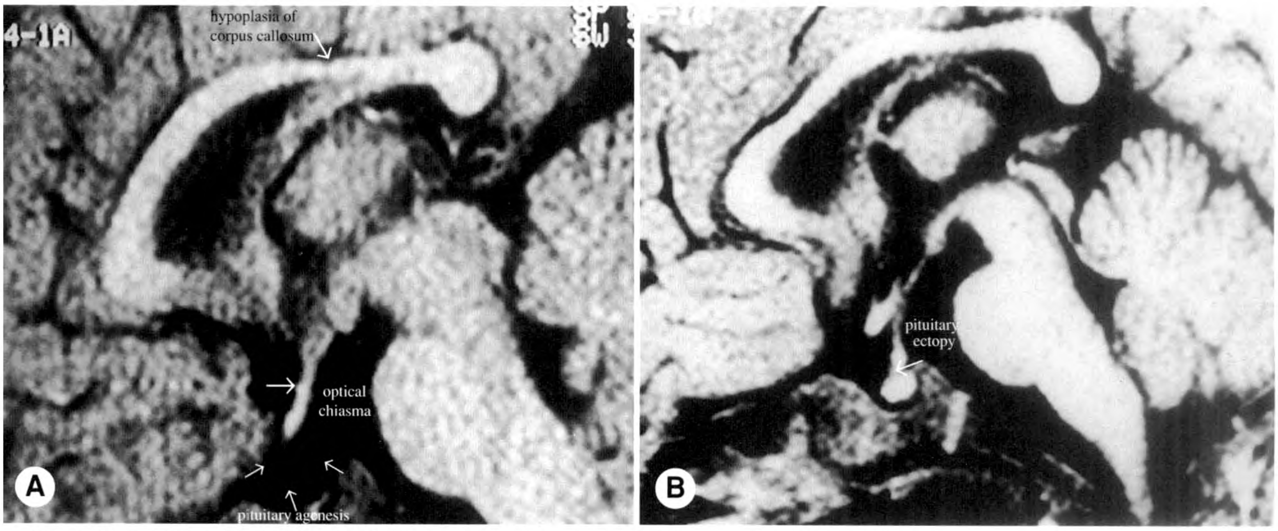


Fig 2. Magnetic resonance T1-weighted images of 2 cases of pituitary abnormalities. A) (Case 4) Total pituitary agenesis. Note optical chiasma and absence of antehypophyseal and post-hypophyseal magnetic resonance signals. B) (Case 7) Ectopia of posterior pituitary lobe. Note spontaneous post-hypophyseal hypersignal above sella.

sence of an upper labial frenulum and intermaxillary suture, and the presence of a medial upper gingival bud (Fig 1A). A CT scan confirmed the presence of CNPAS and a single medial incisor bud (Fig 3). A sublabial approach was then used to install nasal stenting, which was secured in place for 1 week. The nasal airway was greatly improved after removal of the stents. Follow-up at 18 months of age revealed a good nasal airway and the eruption of a central single upper maxillary incisor. His 10-year-old brother had been treated as a neonate for a bilateral choanal atresia by a transnasal approach and prolonged nasal stenting and had presented a central upper incisor without pyriform aperture stenosis.

Case 6. A male infant, the product of a 38-week normal pregnancy, had a birth weight of 2.9 kg. The mother had a history of several spontaneous abortions, and a previous male infant had been stillborn without diagnosis. Despite local treatment with epinephrine drops, he had a persistent nasal obstruction with feeding difficulties after 3 months. Clinical examination revealed narrow nostrils, a flat nasal bridge, and mild hypotelorism. There was no clinical evidence of a central maxillary incisor bud. A CT scan confirmed CNPAS without dental abnormalities, and

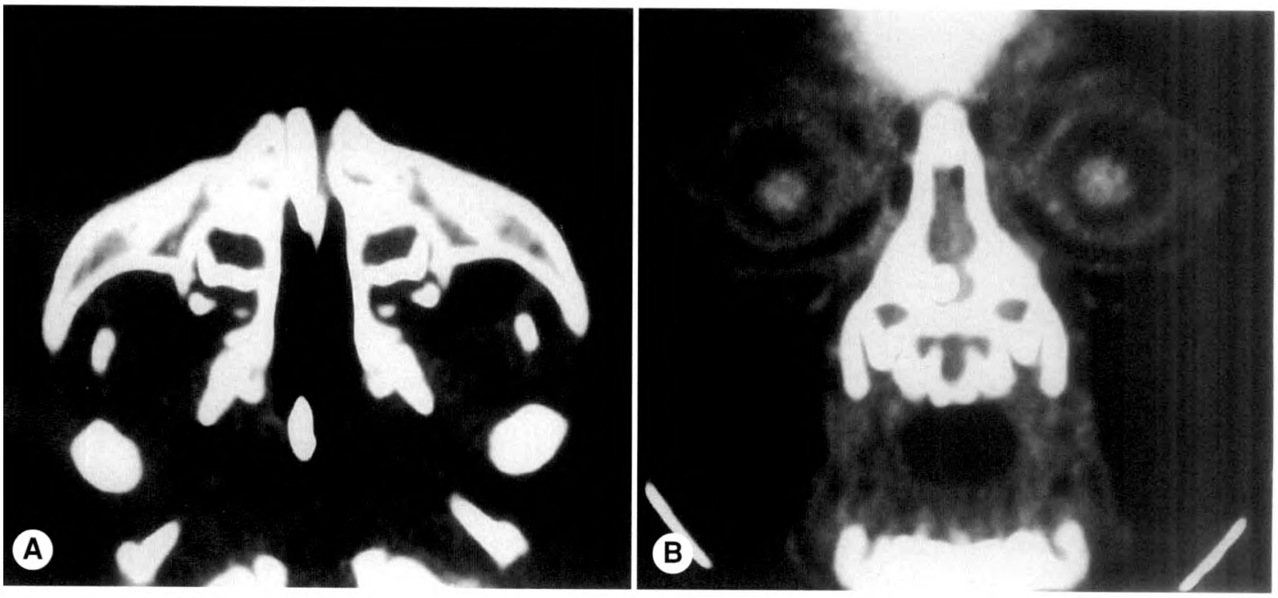


Fig 3. (Case 7) Computed tomograms from child presenting with typical CNPAS. A) Noncontrast bone window axial scan. Note anterior bony narrowing of nasal airway with collapse of nasal valve. Hyperdensity corresponds to nasogastric gavage catheter. B) Bone window anterior coronal section. Note important narrowing of bony aperture as compared with gavage catheter and presence of medial incisor.

MRI showed hypoplasia of the corpus callosum. A sublabial approach was used to insert bilateral nasal stents, which were kept in place for 1 week (3.5-mm endotracheal tubes). His feeding difficulties were reduced. At 1-year follow-up, he was found to be developmentally retarded. His karyotype was normal.

Case 7. A female infant, the product of a 38-week gestation, had a birth weight of 3.2 kg. She developed an immediate obstructive respiratory distress that was relieved by crying. She was treated with a McGovern nipple, local drops of epinephrine, and gavage feeding for 3 days and was then referred to our department to rule out choanal atresia. Clinical examination revealed narrow nostrils, and it was difficult to insert a small probe. Mouth examination revealed the absence of an upper labial frenulum and intermaxillary suture, and the presence of a medial upper gingival bud. A CT scan confirmed the presence of CNPAS and a single medial incisor bud. A sublabial enlargement of the nasal pyriform aperture was performed 10 days later, because the oral cannula could not be removed. Endotracheal tube nasal stents (3-mm and 3.5-mm inner diameter) were used for 1 week. The patient began normal oral feeding immediately after this procedure. An MRI scan revealed an ectopia of the posterior pituitary lobe without other brain abnormality (Fig 2B). The results of endocrinologic function tests were normal. At 1-year follow-up, she was growing normally, her nasal permeability was normal, and she had developed a single central upper incisor.

DISCUSSION

Nasal obstruction is a cause of respiratory distress in newborns, because they are obligate nasal breathers. This condition is temporary, but may occur in children when they are a few days to several weeks old. The classic diagnosis is a bilateral posterior choanal atresia, or edema of the nasal mucosa. But CNPAS has been described recently as a new and rare cause of nasal airway obstruction.[1-3,5] Some recent reports have suggested that there is a relationship between CNPAS and moderate forms of holoprosencephaly, and that CNPAS is a disorder of induction of the cephalic mesoderm by the cephalic notochord.[2,3,5] Several lines of evidence support this hypothesis. First, the disorder is frequently associated with a single upper medial incisor (12 children out of 20 in our series). This malformation has been widely described as a manifestation of microforms of holoprosencephaly.[6] Hypotelorism and a flat nasal bridge are other clinical features of premaxillary dysgenesis associated with holoprosencephaly, and were found in 8 of our cases. The association of CNPAS and a medial upper incisor with typical Apert's syndrome (case 3), another type of induction disorder, reinforces the link between CNPAS with developmental field defects. Another child presented with CNPAS and a craniosynostosis (case 15). Second, it appears to be associated with diencephalic abnormalities. Two of our patients had pituitary abnormalities, and 1 patient had an abnormal corpus callosum. Dental and facial abnormalities have been associated with pituitary dysfunction,[4,7] suggesting a common developmental defect of the prosencephalic derivatives, as in the case of holoprosencephaly. However, the absence of a central maxillary incisor does not exclude the possibility of a midbrain malformation, because 1 patient out of 3 in our series had no dental abnormalities. Whereas most cases of holoprosencephaly are idiopathic, there have been several reports of autosomal or X-linked transmission or chromosomal abnormalities.[8] In our series, a karyotype was made in 7 cases and was normal in all cases, including the 5 children with a familial history or a consanguinity. We found no evidence of an association with maternal drug intake or fetal alcohol syndrome. The mother of patient 11 suffered from insulin-dependent diabetes, a condition previously associated with holoprosencephaly.[8]

Previous reports have indicated several strategies for treatment. Brown et al[1] managed 5 of 6 children surgically, whereas Arlis and Ward[2] managed all 6 of the children they treated conservatively with local topical drops or nasal stenting, and Hui et al[3] limited surgery to failure of medical or stenting management (2 cases out of 6). We believe that the initial management of nasal obstruction in neonates should avoid nasal stenting (see case 1 with necrosis of the septum and columella), but include corticosteroid and epinephrine topical drops. McGovern nipples are indicated for cases of respiratory distress, and gavage feeding is often indispensable for maintaining normal growth. A single central megaincisor can be clinically suspected, even in neonates, when the medial upper labial frenulum and the intermaxillary suture are absent and a medial upper gingival bud is present (Fig 1A). A CT scan must then be performed as soon as possible to confirm the diagnosis and detect other midline abnormalities. In all our cases, the CT scan showed the typical image of anterior narrowing of the bony aperture (Fig 3). The absence of clinical or radiological associated abnormalities of the midline does not eliminate pituitary gland or midbrain abnormalities, and thus MRI and/or endocrinologic testing should be performed early.

The main problem is how to decide whether patients are not responding to medical treatment and should undergo surgery. A mouth cannula should not be kept in place more than 1 week, so as to avoid mucosal ulcerations. Weight loss and failure to thrive are not sensitive indicators, because of the systematic use of gavage feeding. Epinephrine drops and corticosteroids should also not be used more than 10 to 15 days, so as to avoid metabolic side effects (see case 5). In case of a lack of efficacy and/or recurrence of nasal obstruction after 10 to 15 days of medical therapy, surgical enlargement of the nasal pyriform aperture should be considered. The nasal stenting should be kept in place a minimum of 5 days to allow the mucosa to become attached to the new bony margins of the pyriform aperture and to avoid recurrent stenosis, but should not exceed 15 days, so as to avoid mucosal injuries. Epinephrine or corticosteroid drops are often necessary during the week after the stents have been removed. Our attempts to reduce the stenting duration (cases 11 to 13) were unsuccessful in 2 cases and led to a second procedure in case 11 and a more prolonged stenting in case 12.

Analysis of the Table reveals 2 groups of children. The first group consists of 7 children who underwent operation during the first month after birth. Five of them (70%) presented with immediate respiratory distress. In this group, the decision for surgery was based on the ineffectiveness of medical therapy. The second group contains the 13 other children who underwent operation later (range, 1 month to 1 year; mean, 3 months). Only 5 of these children (37%) presented with immediate respiratory distress. They usually responded to initial medical therapy, but remained symptomatic even after a long period of treatment and/or required repeated or extended corticosteroid or epinephrine therapy. The surgical procedure of bony aperture enlargement is relatively easy to perform and, in our hands, gave immediate results. No septal or columellar necrosis was induced by the nasal stents after enlargement of the pyriform aperture. Only 2 young children had a failure of treatment: 1, probably because the stenting duration was too short and the child required a second procedure, and the other, because the stenting duration was too short.

Since 1997, 12 new cases have successfully undergone operation. No recurrence was found in the 20 cases reported in this study.

In conclusion, CNPAS has been reported to be an unusual cause of nasal obstruction in neonates and infants, but its frequency is probably underestimated. A link between CNPAS and holoprosencephaly is supported by the frequency of midline and midbrain abnormalities, and this justifies MRI and/or metabolic and genetic investigations. We believe that surgery is indicated in cases of severe and symptomatic CNPAS, as it shortens the duration of hospitalization and prevents the complications of prolonged medical therapy and stenting. Early surgery is indicated when inital conservative methods fail. However, delayed surgery is often required in case of dependence on epinephrine or corticosteroids. A multidisciplinary approach is also required, including pediatricians specializing in genetic, endocrinologic, and neurologic disorders.

REFERENCES

1. Brown OE, Myer CM, Manning SC. Congenital nasal pyriform aperture stenosis. Laryngoscope 1989;99:86-91.
2. Arlis H, Ward RF. Congenital nasal pyriform aperture stenosis. Isolated abnormality vs developmental field defect. Arch Otolaryngol Head Neck Surg 1992;118:989-91.
3. Hui Y, Friedberg J, Crysdale WS. Congenital nasal pyriform aperture stenosis as a presenting feature of holoprosencephaly. Int J Pediatr Otorhinolaryngol 1995;31:263-74.
4. Beregszaszi M, Leger J, Garel C, et al. Nasal pyriform aperture stenosis and absence of the anterior pituitary gland: report of two cases. J Pediatr 1996;128:858-61.
5. Tavin E, Stecker E, Marion R. Nasal pyriform aperture stenosis and the holoprosencephaly spectrum. Int J Pediatr Otorhinolaryngol 1994;28:199-204.
6. DeMyer W, Zeman W, Palmer CG. The face predicts the brain: diagnostic significance of median facial anomalies for holoprosencephaly (arhinencephaly). Pediatrics 1964;34:256-63.
7. Rappaport E, Ulstrom RA, Gorlin RJ, Lucky AW, Colle E, Miser J. Solitary maxillary central incisor and short stature. J Pediatr 1977;91:924-8.
8. Cohen MM. Perspectives on holoprosencephaly: part I. Epidemiology, genetics and syndromology. Teratology 1989;40:211-35.

Ann Otol Rhinol Laryngol 110:2001

IMMUNOHISTOCHEMICAL ANALYSIS OF SMALL CELL CARCINOMA OF THE HEAD AND NECK: A REPORT OF FOUR PATIENTS AND A REVIEW OF SIXTEEN PATIENTS IN THE LITERATURE WITH ECTOPIC HORMONE PRODUCTION

HIROYUKI MINETA, MD
KATSUTOSHI MIURA, MD SATORU TAKEBAYASHI, MD KEISUKE ARAKI, MD
YOH UEDA, MD HIDEKAZU HARADA, MT KIYOSHI MISAWA, MD
HAMAMATSU, JAPAN

Small cell carcinoma (SCC) occurs mostly in the lung, and in some patients is accompanied by production of ectopic hormones. Small cell carcinoma of the head and neck is very rare. We report 4 patients with SCC of the head and neck (larynx, tonsil, maxillary sinus, and parotid gland). The patient with SCC of the maxillary sinus demonstrated a high level of plasma serotonin and overexpression of parathyroid hormone; however, he did not show any related symptoms. The patient with SCC of the tonsil showed the syndrome of inappropriate secretion of antidiuretic hormone associated with antidiuretic hormone hyperproduction at the terminal stage. In the literature, 16 patients with SCC of the head and neck with ectopic hormone production have been reported. Antidiuretic hormone and adrenocorticotropic hormone were the hormones that caused clinical symptoms (paraneoplastic syndromes). We believe that the evaluation of hormonal syndromes is valuable for diagnosis and treatment.

KEY WORDS — head and neck, hormone, immunohistochemistry, small cell carcinoma.

INTRODUCTION

Neuroendocrine neoplasms are divided into 2 groups: 1) the neural type, including neuroblastoma, pheochromocytoma, and paraganglioma, and 2) the epithelial type, including carcinoid, atypical carcinoid, and small cell carcinoma (SCC).[1] Small cell carcinoma is thought to develop from the argyrophilic Kulchitsky cells or from the amine precursor uptake and decarboxylation cell system.[2] However, the term neuroendocrine does not imply an embryological origin from the neuroectoderm, but rather, connotes a shared phenotype characterized by the simultaneous expression of a wide variety of neuronal and endocrine traits.[3] Small cell carcinoma occurs most commonly in the lung, and accounts for 20%[4] of all lung carcinomas. It is characterized by very rapid local progression and metastatic spread, with a median survival time for untreated patients of only 2 to 3 months.[4] Ultrastructural evidence of neurosecretory granules or use of immunohistochemical reactions is sometimes needed to confirm the diagnosis, and to distinguish SCC from squamous cell carcinoma, adenocarcinoma, and malignant lymphoma. Although extrapulmonary SCC is rare, accounting for 4% of all SCCs,[5] SCC of the head and neck has been reported in the literature: approximately 160 patients had SCC in the larynx,[6-8] 44 in the major salivary glands,[9] 34 in the paranasal sinus and the nasal cavity,[9,10] and 6 in the tonsil.[11]

Small cell carcinoma is known to produce ectopic hormones, and some patients manifest hormone-related symptoms (paraneoplastic syndromes). Among them, the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) is clinically the most important. It is recognized in approximately 10%[12] of patients with pulmonary SCC, which accounts for 75%[4] of tumor-associated SIADH. Ectopic hormone production from head and neck cancers is uncommon, and 78.6%[8] of cases of SIADH associated with head and neck cancers have been reported to originate from squamous cell carcinomas. However, the frequency of ectopic hormone production and of paraneoplastic syndromes is higher in SCC than in squamous cell carcinoma.

In this study, we report 4 patients with SCC of the head and neck and analyze a variety of hormones and peptides using immunohistochemical methods and transmission electron microscopy (TEM), and we review the patients previously reported in whom ectopic production of hormone or peptide was detected.

PATIENTS AND METHODS

Patients. Four cases of SCC of the head and neck

From the Department of Otolaryngology (Mineta, Takebayashi, Araki, Ueda, Misawa) and the Division of Pathology (Miura, Harada), Hamamatsu University School of Medicine, Hamamatsu, Japan. This work was supported by Grant-in-Aid for Scientific Research (C 09671741) to H.M.

CORRESPONDENCE — Hiroyuki Mineta, MD, Dept of Otolaryngology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu, 431-3192, Japan.

were diagnosed from October 1979 to December 1997 at Hamamatsu University Hospital (Hamamatsu, Japan). The TNM classifications were made according to the 1997 International Union Against Cancer criteria.[13] Tissue specimens were collected at biopsy or surgery before any treatment. We performed immunohistochemical analysis with the following markers, all from Dako, Copenhagen, Denmark, for the diagnosis of SCC: keratin (1:300), vimentin (1:3), epithelial membrane antigen (1:100), S-100 protein (1:200), leukocyte common antigen (LCA; 1:100), neuron-specific enolase (NSE; 1:100), chromogranin A (1:100), and synaptophysin (1:10).

Immunohistochemical Analysis of Hormones and Peptides. Five-micrometer sections were dewaxed with xylene, hydrated through graded alcohol, and rinsed in distilled water. The sections were put in a microwave oven in a citrate buffer (pH 6.0) for three 5-minute sessions, cooled to room temperature gradually, and rinsed in distilled water and phosphate buffered saline solution (PBS). Rabbit serum (20%) was applied to the sections for 10 minutes as a blocking reagent to reduce nonspecific binding. The following primary antibodies to hormones and peptides, from Dako, were used: adrenocorticotropic hormone (ACTH; 1:100), human growth hormone (diluted antibody), prolactin (diluted antibody), insulin (1:250), glucagon (1:250), somatostatin (1:250), thyroglobulin (1:100), calcitonin (1:250), serotonin (1:100), and gastrin (1:200). We also used parathyroid hormone (PTH; 1:5, Milab, Malmö, Sweden), antidiuretic hormone (ADH; 1:2,000, ICN, Tokyo, Japan), and vasoactive intestinal polypeptide (diluted antibody, Immulok, Raritan, NJ). The sections were incubated at room temperature for 1 hour. They were rinsed in PBS and incubated with a biotinylated secondary antibody for 30 minutes, followed by incubation with streptavidin peroxidase reagent (StreptABComplex, Dako) for 30 minutes. After washing in PBS, they were incubated in a diaminobenzidine solution for 5 minutes, washed in tap water for 10 minutes, and then counterstained with Mayer's hematoxylin.

Transmission Electron Microscopy. Transmission electron microscopic study was performed in 3 patients for the detection of neurosecretory granules. The specimens for TEM were fixed in 2% phosphate buffered glutaraldehyde overnight, rinsed in 0.1-mol/L phosphate buffer (pH 7.4), post-fixed in 1% phosphate buffered osmium tetroxide, dehydrated in alcohol and propylene oxide, and embedded in epoxy resin. Semithin sections (about 1 µm thick) were stained with toluidine blue and examined by light microscopy. Thin sections from selected areas were stained with uranyl acetate and lead citrate, and were examined by TEM.

RESULTS

Patient Evaluation. Table 1[13] summarizes the 4 cases in this study. Although all of the tumors were advanced, showing massive regional lymph node metastasis, no patients had distant metastasis, and none complained of symptoms associated with hormone or peptide hyperproduction at the first evaluation. The NSE levels in plasma were high in all patients during the course of the disease. Patient 2 revealed SIADH (plasma sodium, 118 mEq/L; plasma osmolarity, 253 mOsm/kg H_2O; urine sodium, 66 mEq/L) with bone metastasis, followed by the elevation of plasma NSE to 43.5 ng/mL (normal, below 10 ng/mL). Patient 3 had a high level of plasma serotonin (590 ng/mL; normal, below 230 ng/mL); however, no related symptoms were present. All patients were treated by chemotherapy with a combination of cisplatin, etoposide, cyclophosphamide, and Adriamycin and by radiotherapy. Patient 4 was treated with a radical operation followed by radiotherapy and chemotherapy. All patients died of distant metastasis (brain, bone, lung, liver, and skin). Patient 4 also had a local recurrence. The follow-up period was 9 to 12 months (mean, 10 months).

Conventional Histologic Analysis. Small cell carcinoma was located mainly in submucosa. Hematoxylin and eosin staining revealed that the tumor was composed of small undifferentiated cells with relatively large hyperchromatic oval, round, or spindle-shaped nuclei and scant cytoplasm. Mitosis and local invasion, including vascular, perineural, and skeletal muscle infiltration, were common (see Figure, A). Patients 1 and 3 were initially given a tentative diagnosis of malignant lymphoma, and patient 4 was given a diagnosis of undifferentiated carcinoma. Epithelial membrane antigen was positive in patients 1, 2, and 4, but none showed immunostaining positive for keratin, vimentin, S-100 protein, or LCA (see Figure, B). Neuron-specific enolase was positive in all patients (see Figure, C), chromogranin A in patients 2 and 3 (see Figure, D), and synaptophysin in patient 3.

Immunohistochemical Analysis of Hormones and Peptides and TEM. The tumor cells reacted with anti-PTH in patient 3 (see Figure, E), but no other antibodies showed an obvious reaction (Table 1). Patient 3, who demonstrated a high level of plasma serotonin, did not show positive immunostaining for serotonin. Patient 2, who showed SIADH later, did not demonstrate ADH immunohistochemical positivity. Transmission electron microscopy demonstrated dense core granules in the cytoplasm in patients 1,

TABLE 1. CHARACTERISTICS OF PATIENTS WITH SMALL CELL CARCINOMA OF HEAD AND NECK

	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*
Age (y)	72	55	55	80
Sex	M	M	M	M
Symptom	Neck mass	Sore throat	Nasal bleeding	Neck mass
Primary site	Larynx (subglottic)	Tonsil	Maxillary sinus	Parotid gland
TNM*	T2N2aM0	T2N2bM0	T4N1M0	T2N2aM0
NSE at first evaluation	9.2 ng/mL	8.9 ng/mL	11.2 ng/mL	8.6 ng/mL
Plasma hormone level	–	–	Serotonin (590 ng/mL)	–
Related syndrome	–	SIADH	–	–
Treatment	Chemotherapy (CDDP + VP16), irradiation	Chemotherapy (CDDP + VP16), irradiation	Irradiation, chemotherapy (CYC + ADM + VCR) (CDDP + VP16)	Operation, irradiation, chemotherapy (CDDP + VP16)
Follow-up	DOD (12 mo)	DOD (10 mo)	DOD (8 mo)	DOD (11 mo)
Pathology				
Tentative	Malignant lymphoma	Small cell carcinoma	Malignant lymphoma	Undifferentiated carcinoma
Final	Small cell carcinoma	Small cell carcinoma	Small cell carcinoma	Small cell carcinoma
IHC				
Keratin	–	–	–	–
Vimentin	–	–	–	–
EMA	+	+	–	+
S-100 protein	–	–	–	–
LCA	–	–	–	–
NSE	+	+	+	+
Chromogranin A	–	+	+	–
Synaptophysin	–	–	+	–
TEM	Granules	Granules	Granules	Not done
Hormonal IHC				
ACTH	–	–	–	–
hGH	–	–	–	–
Prolactin	–	–	–	–
Insulin	–	–	–	–
Glucagon	–	–	–	–
Somatostatin	–	–	–	–
Thyroglobulin	–	–	–	–
Calcitonin	–	–	–	–
Serotonin	–	–	–	–
PTH	–	–	+	–
ADH	–	–	–	–
VIP	–	–	–	–

NSE — neuron-specific enolase; SIADH — syndrome of inappropriate secretion of antidiuretic hormone; CDDP — cisplatin; VP16 — etoposide; CYC — cyclophosphamide; ADM — Adriamycin; VCR — vincristine; DOD — dead of disease; IHC — immunohistochemistry; EMA — epithelial membrane antigen; LCA — leukocyte common antigen; TEM — transmission electron microscopy; ACTH — adrenocorticotropic hormone; hGH — human growth hormone; PTH — parathyroid hormone; ADH — antidiuretic hormone; VIP — vasoactive intestinal polypeptide.

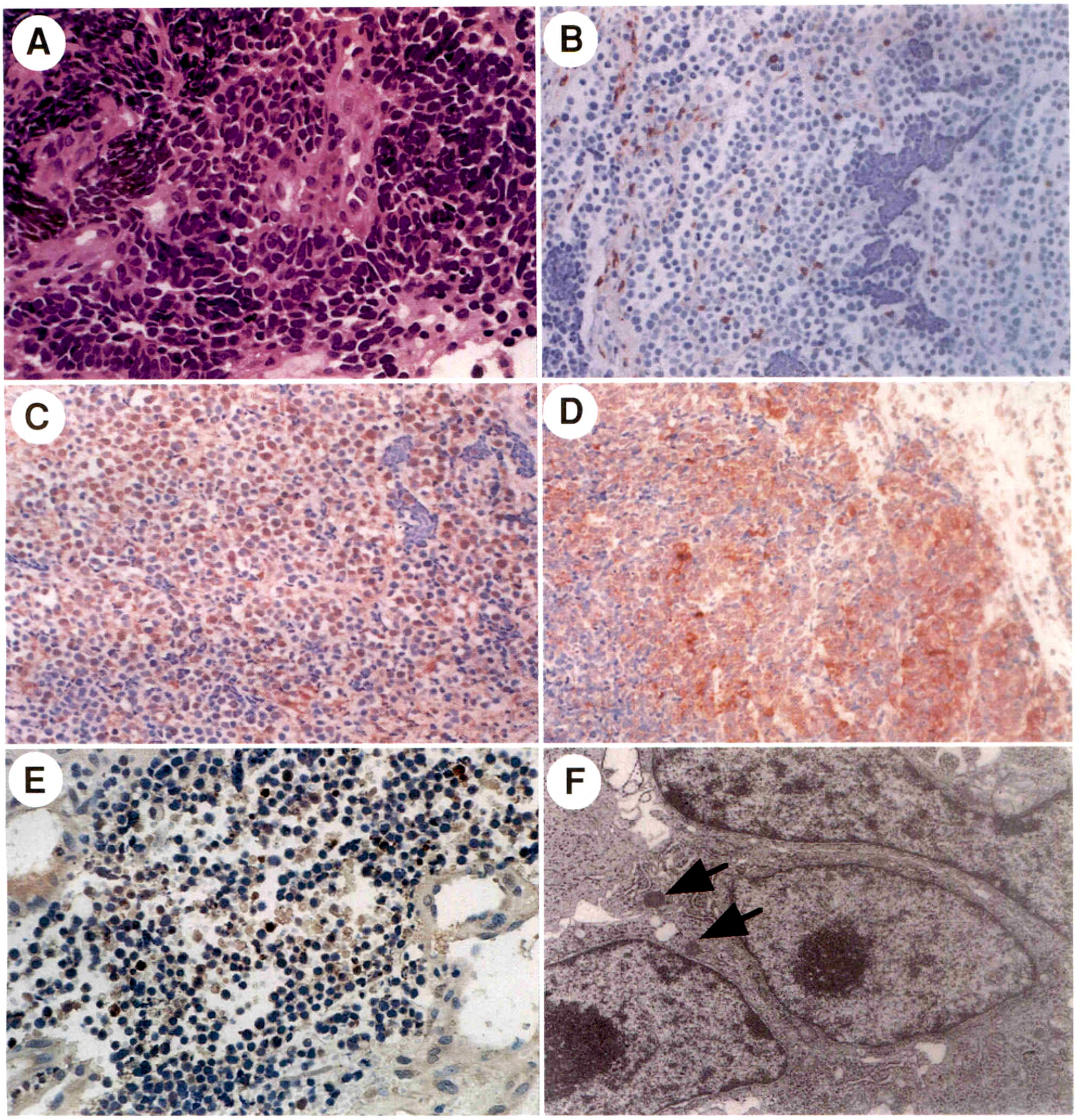
*Classified according to 1997 International Union Against Cancer criteria.[13]

2, and 3 (see Figure, F).

DISCUSSION

We have reported 4 patients with SCC of the head and neck and reviewed 16 patients in the literature with SCC of the head and neck with ectopic hormone production. Hormonal analysis seems to be valuable for diagnosis and treatment.

The diagnosis of SCC needs the support of immunohistochemical or ultrastructural studies. Three of our 4 patients were given a tentative pathological diagnosis based only on hematoxylin and eosin staining: malignant lymphoma in 2 patients, and undifferentiated carcinoma in 1. Small round cell tumors show similar morphology on routine hematoxylin and eosin section. This study indicates that LCA staining is use-



(Patient 3) Histologic findings of patient with small cell carcinoma of maxillary sinus. A) Hematoxylin and eosin (original ×100). Tumor cells had small, round nucleus and scant cytoplasm. Single cell necrosis was frequently seen. B) Leukocyte common antigen (original ×50). Tumor cells were negative. Positive cells were infiltrating lymphocytes. C) Neuron-specific enolase (original ×50). Most tumor cells were positive. D) Chromogranin A (original ×50). Most tumor cells were positive. E) Parathyroid hormone (original ×50). Some few tumor cells were positively stained in cytoplasm. F) Transmission electron microscopy demonstrates small round and dense core neurosecretory granules (arrows) surrounded by limiting membrane (original ×3,000).

ful in distinguishing SCC from malignant lymphoma, and that NSE is also of value in the diagnosis of SCC. Transmission electron microscopy can demonstrate a neuroendocrine tumor directly, if electron-dense neurosecretory granules are identified in the tumor cytoplasm. Three patients revealed small, round, and dense core neurosecretory granules surrounded by limiting membrane. A case with a histopathologic finding of small round cells having scant cytoplasm and not arranged in any specific pattern should be submitted to LCA and NSE immunostaining, followed by chromogranin A or synaptophysin immunostaining, or ultrastructural analysis for detecting neurosecretory granules in tumor cells. Also, proprotein convertases (PCs), which transform into active protein, have been described.[1] Antibodies against PCs,

TABLE 2. SUMMARY OF REPORTED PATIENTS WITH SMALL CELL CARCINOMA OF HEAD AND NECK WITH ECTOPIC HORMONE PRODUCTION

Patient No. and Reference	*Age (y)/ Sex*	*Primary Site*	*Hormones*	*Method*	*Related Syndrome*	*TEM*	*Treatment*	*Follow-up*
1[25]	57/M	Parotid gland	ACTH	NE	Cushing's disease	Granules	Operation	DOD, 16 mo
2[15]	61/M	Larynx (subglottic)	ADH	BA	SIADH	ND	Radiotherapy	DOD, 7.7 mo
3[24]	50/M	Paranasal sinus	ACTH	RIA	NE	Granules	Operation	DOD, 10 y
4[24]	56/M	Paranasal sinus	ACTH, β-MSH	RIA	NE	Granules	Operation	DOD, 6 y
5[24]	70/M	Paranasal sinus	Calcitonin	RIA	NE	Granules	Chemotherapy, radiotherapy	DOD, 6 mo
6[16]	59/M	Larynx	Calcitonin	IHC	NE	Granules	ND	DOD, 18 mo
7[16]	61/F	Larynx	Calcitonin, somatostatin, ACTH	IHC	NE	Granules	Operation	NED, 8 mo
8[17]	64/F	Larynx (supraglottic)	Unknown	ND	Eaton-Lambert syndrome	Granules	Chemotherapy, radiotherapy	DOD, 11 mo
9[18]	51/M	Larynx (supraglottic)	Serotonin	IHC	NE	ND	Operation, chemotherapy, radiotherapy	DOD, 15 mo
10[19]	60/F	Larynx (supraglottic)	ACTH, GRP, calcitonin, β-endorphin	IHC	Cushing's disease	Granules	Radiotherapy	DOD
11[20]	42/NE	Larynx (subglottic)	Serotonin	IHC	NE	Granules	Operation, chemotherapy	AWD, 16 mo
12[21]	NE	Larynx	Bombesin	IHC	NE	ND	Not reported	Not reported
13[22]	77/F	Larynx (supraglottic)	Serotonin	IHC	NE	ND	Operation	NED, 7 mo
14[23]	53/M	Larynx (supraglottic)	ADH	Na, Osm	SIADH	No granules	Radiotherapy, operation, chemotherapy	DOD, 15 mo
15[9]	51/M	Paranasal sinus	ADH	Na, Osm	SIADH	ND	Chemotherapy	AWD, 16 mo
16[7]	58/M	Larynx (supraglottic)	ADH	Na, Osm	SIADH	Granules	Chemotherapy	DOD
This study	55/M	Tonsil	ADH	Na, Osm	SIADH	Granules	Chemotherapy	DOD, 10 mo
	55/M	Maxillary sinus	Serotonin, PTH	RIA, IHC	NE	ND	Radiotherapy, chemotherapy	DOD, 8 mo

TEM — transmission electron microscopy; ACTH — adrenocorticotropic hormone; NE — no evidence; DOD — dead of disease; ADH — antidiuretic hormone; BA — biological assay; SIADH — syndrome of inappropriate secretion of antidiuretic hormone; ND — not done; RIA — radioimmunoassay; β-MSH — β-melanocyte–stimulating hormone; IHC — immunohistochemistry; NED — no evidence of disease; GRP — gastrin-releasing peptide; AWD — alive with disease; Na — plasma and/or urine sodium concentration; Osm — plasma and/or urine osmolarity; PTH — parathyroid hormone.

especially PC1/PC3 and PC2, have been used as neuroendocrine cell markers.

However, NSE has cross-reactivity in many non-neuroendocrine tumors because of the various isoforms of enolase, detracting from its utility as a specific marker. All patients demonstrated NSE-positive immunostaining, although only 1 patient showed a slight elevation of plasma NSE (11.2 ng/mL) at the first evaluation. Similarly, in the produced hormones, differences between the results of immunohistochemical analysis and plasma levels existed. Patient 3 revealed an elevation of plasma serotonin (590 ng/mL), but no positive immunostaining for serotonin. Although patient 2 revealed SIADH in the terminal stage of SCC, no ADH positivity was present in the specimen for immunohistochemical analysis that was taken at the first evaluation. These discrepancies reflect the relative sensitivity of the specific epitopes recognized by the two analyses, and reflect the period of sampling or analyzing. In addition, immunohistochemical analysis cannot reveal how much hormone the tumor cells secrete to extracellular regions.

Ectopic hormone production occurs much less frequently in SCC of the head and neck than in pulmonary SCC. Antidiuretic hormone, ACTH, calcitonin, PTH, glucagon, and somatostatin[4,14] are usually detected in pulmonary SCC. We presented 2 patients with ectopic hormone production: ADH in patient 2, and serotonin and PTH in patient 3. To date, only 16 patients with SCC of the head and neck with ectopic hormone production have been reported, including 11 patients with SCC of the larynx,[7,15-23] 4 of the paranasal sinus,[9,24] and 1 of the parotid gland[25] (Table 2). The most commonly secreted hormones were ACTH in 5 patients, ADH in 4, calcitonin in 4, and serotonin in 3. Three patients showed production of 2 or 3 hormones.

Approximately half of the patients who had hormone secretion demonstrated related symptoms. Antidiuretic hormone and ACTH are the main hormones to cause hormone-related syndromes. In this study, patient 2 showed SIADH due to ectopic ADH production, and patient 3, with an elevation of plasma serotonin and PTH immunohistochemical positivity, did not show any related syndrome. Seven of the 16 previously reported patients showed related syndromes of hormone hyperproduction clinically, including 4 with SIADH, 2 with Cushing's disease, and 1 with Eaton-Lambert syndrome. The remaining 9 patients did not show any syndromes. These secreted hormones had a low level of function, or none, resulting from aberrant processing of messenger RNA.[4] Secretion of a nonfunctional hormone would not cause a syndrome that is clinically recognized. Similar to pulmonary SCC, SCC of the head and neck demonstrates SIADH and Cushing's disease associated with ectopic hormone production.

The prognosis of SCC of the head and neck is very poor because of the high incidence of distant metastasis that exists even if the tumor temporarily responds to chemotherapy or radiotherapy. Our 4 patients died of disease, with distant metastases to brain, lung, bone, and skin, and local recurrence in 1 patient. In contrast to the prognosis of pulmonary SCC (1%[26] or 4%[27] 5-year survival rate), one of the most interesting features of SCC of the head and neck is the possibility of prolonged survival.[28] Although the survival rate of SCC of the larynx, the hypopharynx, or the trachea is poor and is similar to that of pulmonary SCC, patients with SCC of the nasal cavity may have a prolonged survival (in 1 report,[29] 100%; these cases were termed neuroendocrine carcinoma and most likely represented a variant of SCC that differed in clinical behavior[30]), as may patients with SCC of the minor salivary glands (30%[31]).

The survival rate of patients with pulmonary SCC with ectopic hormone production is consistently inferior, because they have an increased rate of central nervous system metastasis.[32] Similarly, in SCC of the head and neck, the prognosis of patients with ectopic hormone secretion is poor. Patients 2 and 3 in this study died of disease at 10 and 8 months, respectively, and only 2 of the 16 patients in the literature were alive with no evidence of disease for 7 and 18 months. It does not seem to be useful to survival that the patients with SCC of the head and neck were examined for ectopic hormone production. However, we suppose that analysis of ectopic hormone production is valuable for diagnosis and treatment. Patient 3, whose disease was tentatively diagnosed as malignant lymphoma, was clinically suspected of having SCC because of the ectopic production of PTH. Although the prognosis of patients with ectopic hormone production is poor, early treatment of SIADH or Cushing's disease could provide an improved quality of life, which in turn could increase the survival rate. Also, hormone monitoring is useful in evaluating treatment response and tumor relapse. Because hormone-related syndromes have been observed after chemotherapy[33] or neck dissection,[34] and in patients with recurrent neoplasms,[35] it is important to evaluate hormonal syndromes during the clinical course.

In summary, we reported 4 patients with SCC of the head and neck, and reviewed 16 patients with head and neck SCC reported to have ectopic hormone production. Although the prognosis of patients with SCC of the head and neck is poor, we believe that the analysis of ectopic hormone production is valuable for diagnosis and treatment.

ACKNOWLEDGMENTS — We thank K. Ishikawa and Y. Mouri for their technical support in immunohistochemistry.

REFERENCES

1. Lloyd RV. Overview of neuroendocrine cells and tumors. Endocrinol Pathol 1996;7:323-8.

2. Pearse AGE. The cytochemistry and ultrastructure of polypeptide hormone–producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J Histochem Cytochem 1969;17:303-13.

3. DeLellis RA, Dayal Y, Tischler AS, Lee AK, Wolfe HJ. Multiple endocrine neoplasia (MEN) syndromes: cellular origins and interrelationships. Int Rev Exp Pathol 1986;28:163-215.

4. Dearnaley D. Small-cell lung cancer: report of a meeting of physicians and scientists at the Royal Marsden Hospital. Lancet 1995;345:1285-9.

5. Ibrahim NBN, Briggs JC, Corbishley CM. Extrapulmo-

nary oat cell carcinoma. Cancer 1984;54:1645-61.

6. Ferlito A. Diagnosis and treatment of small cell carcinoma of the larynx: a critical review. Ann Otol Rhinol Laryngol 1986;95:590-600.

7. Myers TJ, Kessimian N. Small cell carcinoma of the larynx and ectopic antidiuretic hormone secretion. Otolaryngol Head Neck Surg 1995;113:301-4.

8. Ferlito A, Rinaldo A, Devaney KO. Syndrome of inappropriate antidiuretic hormone secretion associated with head and neck cancers: review of the literature. Ann Otol Rhinol Laryngol 1997;106:878-83.

9. Pierce S, Cibull ML, Metcalfe MS, Sloan D. Bone marrow metastases from small cell cancer of the head and neck. Head Neck 1994;16:266-71.

10. Chaudhry MR, Akhtar S, Kim DS. Neuroendocrine carcinoma of the ethmoid sinus. Eur Arch Otorhinolaryngol 1994; 251:461-3.

11. Bawa R, Wax M. Small cell carcinoma of the tonsil. Otolaryngol Head Neck Surg 1995;113:328-33.

12. List AF, Hainsworth JD, Davis BW, Hande KR, Greco FA, Johnson DH. The syndrome of inappropriate secretion of antidiuretic hormone (SIADH) in small cell lung cancer. J Clin Oncol 1986;4:1191-8.

13. Sobin LH, Wittekind C, eds. International Union Against Cancer. TNM classification of malignant tumours. 5th ed. New York, NY: Wiley-Liss, 1997.

14. Delisle L, Boyer MJ, Warr D, et al. Ectopic corticotropin syndrome and small-cell carcinoma of the lung: clinical features, outcome, and complications. Arch Intern Med 1993;153: 746-52.

15. Trotoux J, Glickmanas M, Sterkers O, Trousset M, Pinel J. Syndrome de Schwarz-Bartter: révélateur d'un cancer laryngé sous-glottique à petites cellules. Ann Otolaryngol Chir Cervicofac 1979;96:349-58.

16. Gould VE, Banner BF, Baerwaldt M. Neuroendocrine neoplasms in unusual primary sites. Diagn Histopathol 1981;4: 263-77.

17. Medina JE, Moran M, Goepfert H. Oat cell carcinoma of the larynx and Eaton-Lambert syndrome. Arch Otolaryngol 1984;110:123-6.

18. Woodruff JM, Shah JP, Huvos AG, Erlandson RA, Gerold FP. Neuroendocrine carcinomas of the larynx: a study of two types, one of which mimics thyroid medullary carcinoma. Am J Surg Pathol 1985;9:771-90.

19. Bishop JW, Osamura Y, Tsutsumi Y. Multiple hormone production in an oat cell carcinoma of the larynx. Acta Pathol Jpn 1985;35:915-23.

20. Weidauer H, Blobel GA, Nemetschek-Gansler H, Gould VE, Mall G. Das neuro-endokrine Larynxkarzinom vom kleinzelligen (oat-cell) Typ. Morphologische und immunohistochemische Befunde und ihre Bedeutung für die Therapie. Laryngol Rhinol Otol (Stuttg) 1985;64:121-7.

21. Springall DR, Ibrahim NBN, Rode J, Sharpe MS, Bloom SR, Polak JM. Endocrine differentiation of extrapulmonary small cell carcinoma demonstrated by immunohistochemistry using antibodies to PGP 9.5, neuron-specific enolase and the C-flanking peptide of human pro-bombesin. J Pathol 1986;150: 151-62.

22. Googe PB, Ferry JA, Bhan AK, Dickersin GR, Pilch BZ, Goodman M. A comparison of paraganglioma, carcinoid tumor, and small-cell carcinoma of the larynx. Arch Pathol Lab Med 1988;112:809-15.

23. Takeuchi K, Nishii S, Jin CS, Ukai K, Sakakura Y. Anaplastic small cell carcinoma of the larynx. Auris Nasus Larynx 1989;16:127-32.

24. Kameya T, Shimosato Y, Adachi I, Abe K, Ebihara S, Ono I. Neuroendocrine carcinoma of the paranasal sinus: a morphological and endocrinological study. Cancer 1980;45:330-9.

25. Recant L, Lacy P. Cushing's syndrome associated with a parotid gland tumor. Am J Med 1963;34:394-406.

26. Cook RM, Miller YE, Bunn PA Jr. Small cell lung cancer: etiology, biology, clinical features, staging, and treatment. Curr Probl Cancer 1993;17:71-141.

27. Souhami RL, Law K. Longevity in small cell lung cancer: a report to the Lung Cancer Subcommittee of the United Kingdom Coordinating Committee for Cancer Research. Br J Cancer 1990;61:584-9.

28. Remick SC, Hafez GR, Carbone PP. Extrapulmonary small-cell carcinoma: a review of the literature with emphasis on therapy and outcome. Medicine 1987;66:457-71.

29. Silva EG, Butler JJ, Mackay B, Goepfert H. Neuroblastoma and neuroendocrine carcinomas of the nasal cavity: a proposed new classification. Cancer 1982;50:2388-405.

30. Baugh RF, Wolf GT, McClatchey KD. Small cell carcinoma of the head and neck. Head Neck Surg 1986;8:343-54.

31. Koss L, Spiro RH, Hajdu S. Small cell (oat cell) carcinoma of minor salivary gland origin. Cancer 1972;30:737-41.

32. Lokich JJ. The frequency and clinical biology of the ectopic hormone syndromes of small cell carcinoma. Cancer 1982; 50:2111-4.

33. Hayes DF, Lechan RM, Posner MR, Weichselbaum RR, Miller D, Ervin TJ. The syndrome of inappropriate antidiuretic hormone secretion associated with induction chemotherapy for squamous cell carcinoma of the head and neck. J Surg Oncol 1986;32:150-2.

34. Wenig BL, Heller KS. The syndrome of inappropriate secretion of antidiuretic hormone (SIADH) following neck dissection. Laryngoscope 1987;97:467-70.

35. Talmi YP, Hoffman HT, McCabe BF. Syndrome of inappropriate secretion of arginine vasopressin in patients with cancer of the head and neck. Ann Otol Rhinol Laryngol 1992;101: 946-9.

Ann Otol Rhinol Laryngol 110:2001

NITRIC OXIDE MEDIATES PLATELET ACTIVATING FACTOR–INDUCED MICROVASCULAR LEAKAGE IN RAT AIRWAYS

SEA-YUONG JEON, MD EUN-AH KIM YONG-WOON MA, MD
JIN-PYEONG KIM, MD TAE-GEE JUNG, MD EUI-GEE HWANG, MD
CHINJU, KOREA

Platelet activating factor (PAF), a highly potent chemical mediator in inflammation and allergic reactions, induces microvascular leakage in several tissues. In rat airways, PAF-induced microvascular leakage is probably mediated by an endothelial cell receptor in the microvessels. Nitric oxide (NO), first identified as endothelium-derived relaxing factor, has been suggested to be a mediator of airway microvascular leakage. However, the role of NO in PAF-induced microvascular leakage in the airways has not yet been established. The aim of this study was to investigate the role of NO in PAF-induced microvascular leakage in rat nasal mucosa and trachea. We injected PAF (1 μg/kg) intravenously, and the amount of PAF-induced microvascular leakage was measured with extravasation of Evans blue dye (30 mg/kg, injected intravenously 5 minutes before the injection of PAF) by means of spectrophotometry and fluorescence microscopy. Five Sprague-Dawley rats were pretreated with N^G-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg, intravenously injected 1 hour before the injection of PAF) to inhibit NO synthase, and control rats ($n = 4$) were pretreated with normal saline solution. The average amount of extravasated Evans blue dye was significantly lower in the L-NAME–pretreated rats than in the control rats (*t*-test, $p < .01$). Tissue sections of the L-NAME–pretreated rats clearly showed a decreased extravasation of Evans blue dye on fluorescence microscopy. In conclusion, pretreatment with L-NAME clearly inhibited PAF-induced microvascular leakage in the nasal mucosa and trachea of rats. This finding implies that PAF may activate the constitutive endothelial NO synthase in the microvessels, and that activated endogenous NO may mediate PAF-induced microvascular leakage in rat airways.

KEY WORDS — microvascular leakage, nasal mucosa, nitric oxide, nitric oxide synthase, platelet activating factor, trachea.

INTRODUCTION

Platelet activating factor (PAF), a highly potent inflammatory mediator formed by the action of phospholipase A2 on membrane phospholipids, is one of the major mediators in allergic inflammation. Platelet activating factor constricts the airway smooth muscle, induces microvascular leakage in several tissues, causes the aggregation of inflammatory cells, including eosinophils, and causes epithelial damage in the airways.[1] In guinea pig airways, PAF results in the extravasation of circulating Evans blue dye, which binds to intravascular albumin, throughout the respiratory tract. This PAF-induced airway microvascular leakage is not dependent on arachidonic acid metabolism nor on circulating platelets.[2] The affected vessels are probably postcapillary venules, and PAF may act by means of an endothelial cell receptor in these microvessels.[3]

Nitric oxide (NO) is a gas that transmits signals in the organism. It has now been established that NO, first identified as endothelium-derived relaxing factor, acts as a signal molecule in a variety of important biological systems and has a major role in the regulation of vascular tone, local blood flow and blood pressure, and inhibition of platelet aggregation and adhesion, and has some involvement in postischemic reperfusion.[4] The possible involvement of NO in airway microvascular leakage has been suggested. However, the role of NO in modulating airway microvascular permeability is still controversial. It has been suggested that under physiological conditions, endogenous NO suppresses plasma leakage, but when the induction of NO synthase (NOS) is expressed by lipopolysaccharide (LPS), the increased production of NO enhances plasma leakage.[5] In contrast, pretreatment with an NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), significantly inhibits substance P (SP)–induced and leukotriene D4 (LTD4)–induced extravasation, and L-arginine reverses L-NAME–induced inhibition in guinea pig airways.[6] However, the role of NO in PAF-induced microvascular leakage in the airways has not yet been established.

The aim of this study was to investigate the role of NO in PAF-induced microvascular leakage in rat airways. In this study, the amount of PAF-induced microvascular leakage in the nasal mucosa and trachea was measured with an Evans blue dye assay[2] and assessed with fluorescence microscopy, and the inhibition of PAF-induced microvascular leakage after pretreatment with L-NAME was determined.

From the Department of Otolaryngology, GyeongSang National University Hospital, Chinju, Korea.

CORRESPONDENCE — Sea-Yuong Jeon, MD, Dept of Otolaryngology, GyeongSang National University Hospital, Chilamdong 90, Chinju, 660-702, Korea.

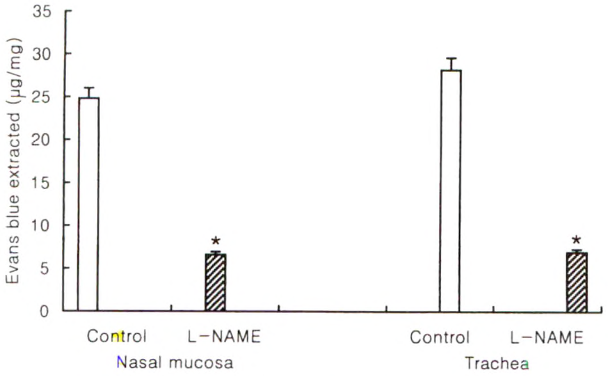


Fig 1. Amount of platelet activating factor–induced extravasated Evans blue dye in nasal mucosa and trachea. Extravasated Evans blue dye is significantly more inhibited in N^G-nitro-L-arginine methyl ester (L-NAME)–pretreated rats than in control rats. * — $p < .01$.

MATERIALS AND METHODS

Animal Preparation. Female Sprague-Dawley rats weighing 250 to 350 g were used. The animals were anesthetized with intraperitoneally administered ketamine hydrochloride (75 mg/kg) and xylazine hydrochloride (10 mg/kg). Five rats were pretreated with L-NAME (10 mg/mL in 0.9% saline solution, 10 mg/kg) injected into a tail vein 1 hour before the injection of PAF. Four control rats were pretreated with 0.9% saline solution. Fifty minutes later, Evans blue dye (30 mg/mL in 0.9% saline solution, filtered with a 5.0-μm Millipore filter, 30 mg/kg) was injected into a tail vein. Five minutes later, PAF (1 μL/mL in 0.25% bovine serum albumin–saline solution, 1 μg/kg) was injected into a tail vein. Five minutes later, the thorax was opened and a catheter was passed through a left ventriculotomy into the aorta. The right atrium was incised, and the animal was perfused with 200 mL of 1% paraformaldehyde in phosphate buffer (PB; 0.1 mol/L, pH 7.2) to remove intravascular dye and to fix the tissue. The entire nasal mucosa, including the septum and turbinates, and the trachea were removed. The wet weight of all tissues was recorded.

Evans Blue Dye Assay. The Evans blue dye was extracted by incubating the tissues in 2 mL of 100% formamide at 60°C for 24 hours, and its concentration was determined by light absorbance at a 620-nm wavelength (Beckman DU series 650, Fullerton, Calif). The amount of Evans blue dye from each sample was expressed in micrograms per gram wet weight of tissue.

Fluorescence Microscopy. To assess the distribution of extravasated Evans blue dye, segments of trachea from an L-NAME–pretreated rat and a control rat were placed in the same fixative at 4°C for 4 hours. The specimens were washed in PB, saturated overnight at 4°C with 25% sucrose in PB, frozen in liquid nitrogen, and cut into sections of 20-μm thickness on a cryostat. The cryostat sections were dried and hydrated in PB and examined under a transmission fluorescence microscope (Olympus BX 50, Tokyo, Japan) at a 435-nm wavelength.

RESULTS

Evans Blue Dye Assay. The average amounts of extravasated Evans blue dye in the nasal mucosa and trachea of the control rats were 23.89 and 28.10 μg per gram of wet tissue, respectively. Those of the L-NAME–pretreated rats were 7.34 and 7.02 μg per gram of wet tissue, respectively (Fig 1). The extravasation of Evans blue dye was significantly inhibited in the L-NAME–pretreated rats as compared with the control rats (*t*-test, $p < .01$).

Fluorescence Microscopy. Extravasated Evans blue dye shows bright orange-red fluorescence under ultraviolet light. The control sections showed intense fluorescence of extravasated Evans blue dye in the subepithelial space and some in the epithelial layer. In contrast, the L-NAME–pretreated sections showed little evidence of fluorescence (Fig 2). The tissue sections of the L-NAME–pretreated rats clearly showed decreased extravasation of Evans blue dye under the fluorescence microscope.

DISCUSSION

We clearly demonstrated that L-NAME, an NOS inhibitor, blocked PAF-induced microvascular leakage in rat airways. The microvascular leakage induced by PAF is endothelium-dependent[2,3] and may be mediated by a biochemical signaling-transduction pathway that involves stimulation of NOS activity.[7] In this study, we investigated the role of NO in PAF-induced microvascular leakage in rat nasal mucosa and trachea by inhibiting NOS. Our findings are in agreement with those of previous reports in which NOS inhibitors blocked PAF-induced microvascular leakage in hamster cheek pouches.[7,8]

The role of NO in modulating microvascular permeability is still controversial and has yet to be established. Inhibition of NO production in rat mesentery leads to an increase in microvascular leakage.[9,10] In contrast, inhibition of NO production in isolated coronary venules[11] and the hamster cheek pouch[12] significantly decreases microvascular leakage. In addition, inhibition of NO production leads to a decreased level of SP- and LTD_4-induced[5] and antigen-induced[13] microvascular leakage in guinea pig airways. Moreover, the inhibition of NO production in LPS-induced microvascular leakage in rat intestine differs according to the time of administration of NOS inhibitors.[14] It has been suggested that the discrepancy between the

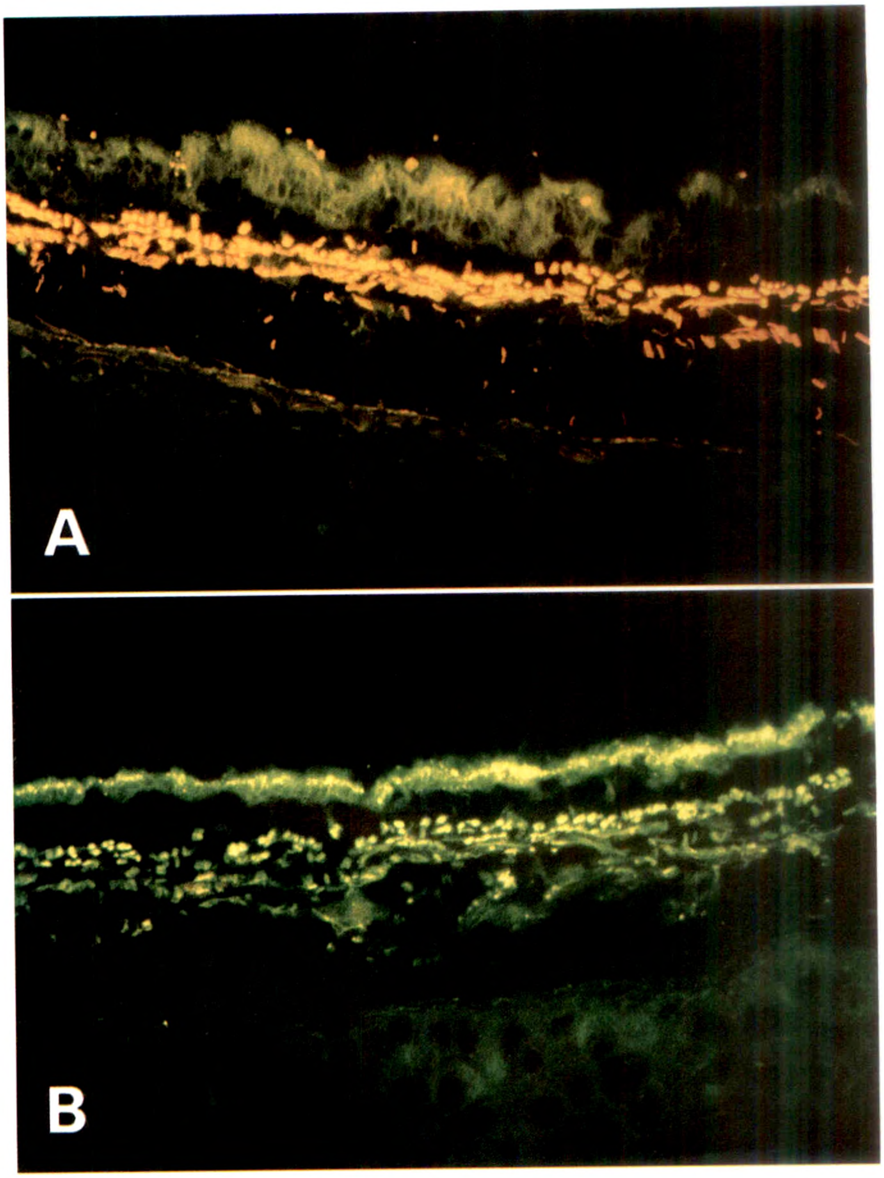


Fig 2. Fluorescence photomicrographs of rat trachea (original ×200). A) Intense orange-red fluorescence of extravasated Evans blue dye is seen in subepithelial space, and some is seen in epithelial layer on control section. B) Fluorescence of extravasated Evans blue dye is hardly observed on L-NAME–pretreated section.

effects of inhibiting NOS may be attributed to the difference in the distribution and role of NOS in different tissues, as well as differences in the route and the time of administration of the NOS inhibitor.[15] It appears that under physiological conditions, endogenous NO may suppress plasma leakage, but under certain pathological conditions induced with PAF, SP, LTD4, or allergen, the activated endogenous NO increases plasma leakage in the airway microvessels.

Nitric oxide synthase exists in several isoforms. Neuronal NOS (nNOS) and endothelial NOS (eNOS) are constitutive (ie, cNOS) and continuously produce endogenous NO. Inducible NOS (iNOS) is not constitutively expressed, but is induced by LPS or certain immunologic stimuli and produces NO for a sustained period.[16] De novo synthesis of iNOS requires about 2 to 4 hours from the time of induction.[17] L-NAME inhibits both cNOS and iNOS. In our study, PAF-induced microvascular leakage occurred in a relatively short time — within 5 minutes. Therefore, L-NAME appears to inhibit cNOS rather than iNOS under our experimental conditions. Our findings imply that PAF may activate the constitutive eNOS in the microvessels, and that activated endogenous NO may mediate PAF-induced microvascular leakage in rat airways.

L-NAME is a potent NOS inhibitor. We observed a dose-dependent inhibition of PAF-induced microvascular leakage with L-NAME in a pilot study performed to determine the dosage of L-NAME to be used in this study. However, L-NAME is not considered an exclusively specific inhibitor of NOS; it could block muscarinic receptors on the vessels and inhibits acetylcholine-induced vasodilation.[18] Further studies with more specific inhibitors of NOS, as well as a more specific inhibitor of eNOS, may help to elucidate the exact role of NO in PAF-induced microvascular leakage.

In conclusion, pretreatment with L-NAME clearly inhibited PAF-induced microvascular leakage in the nasal mucosa and trachea of rats. This finding implies that PAF may activate the constitutive eNOS in the microvessels, and that activated endogenous NO may mediate PAF-induced microvascular leakage in rat airways.

REFERENCES

1. Barnes PJ, Chung KF, Page CP. Platelet-activating factors as a mediator of allergic disease. J Allergy Clin Immunol 1998;81:919-34.

2. Evans TW, Chung K, Rogers DF, Barnes PJ. Effect of platelet-activating factor on airway vascular permeability: possible mechanisms. J Appl Physiol 1987;63:479-84.

3. O'Donnell SR, Barnett CJ. Microvascular leakage to platelet activating factor in guinea-pig trachea and bronchi. Eur J Pharmacol 1987;138:385-96.

4. Furchgott RF. The 1996 Albert Lasker Medical Research Awards. The discovery of endothelium-derived relaxing factor and its importance in the identification of nitric oxide. JAMA 1996;276:1186-8.

5. Bernareggi M, Mitchell JA, Barnes PJ, Belvisi MG. Dual action of nitric oxide on airway plasma leakage. Am J Respir Crit Care Med 1997;155:869-74.

6. Kageyama N, Miura M, Ichinose M, et al. Role of endogenous nitric oxide in airway microvascular leakage induced by inflammatory mediators. Eur Respir J 1997;10:13-9.

7. Ramire MM, Quardt SM, Kim D, Oshiro H, Minnicozzi M, Duran WN. Platelet activating factor modulates microvascular permeability through nitric oxide synthesis. Microvasc Res 1995;50:223-34.

8. Noel AA, Fallek SR, Hobson RW 2nd, Duran WN. Inhibition of nitric oxide synthase attenuates primed microvascular permeability in the in vivo microcirculation. J Vasc Surg 1995;22:661-9.

9. Kubes P, Granger DN. Nitric oxide modulates microvascular permeability. Am J Physiol 1992;262:H611-H615.

10. Kurose I, Kubes P, Wolf R, et al. Inhibition of nitric oxide production: mechanisms of vascular albumin leakage. Circ Res 1993;73:164-71.

11. Yuan Y, Granger HJ, Zawieja DC, Chilian WM. Flow modulates coronary venular permeability by a nitric oxide–related mechanism. Am J Physiol 1992;263:H641-H646.

12. Mayhan WG. Role of nitric oxide in modulating permeability of hamster cheek pouch in response to adenosine 5'-diphosphate and bradykinin. Inflammation 1992;16:295-305.

13. Miura M, Ichinose M, Kageyama N, et al. Endogenous nitric oxide modifies antigen-induced microvascular leakage in sensitized guinea pig airways. J Allergy Clin Immunol 1996;98:144-51.

14. Laszlo F, Whittle BJ, Moncada S. Time-dependent enhancement or inhibition of endotoxin-induced vascular injury in rat intestine by nitric oxide synthase inhibitors. Br J Pharmacol 1994;111:1309-15.

15. Ball SS, Prazma J, Dais D, Rosbe KW, Pillsbury HC. Nitric oxide: a mediator of endotoxin-induced middle ear effusions. Laryngoscope 1996;106:1021-7.

16. Nathan C. Nitric oxide as a secretory product of mammalian cells. FASEB J 1992;6:3051-64.

17. Kolls J, Xie J, LeBlanc R, et al. Rapid induction of messenger RNA for nitric oxide synthase II in rat neutrophils in vivo by endotoxin and its suppression by prednisolone. Proc Soc Exp Biol Med 1994;205:220-9.

18. Chang HY, Chen CW, Hsiue TR. Comparative effects of L-NOARG and L-NAME on basal blood flow and ACh-induced vasodilatation in rat diaphragmatic microcirculation. Br J Pharmacol 1997;120:326-32.

Ann Otol Rhinol Laryngol 110:2001

MICROBIOLOGY OF SEROUS OTITIS MEDIA IN CHILDREN: CORRELATION WITH AGE AND LENGTH OF EFFUSION

ITZHAK BROOK, MD, MSC

PAULA YOCUM, MSC
BRUCE FELDMAN, MD

KIRAN SHAH, MD
STEPHEN EPSTEIN, MD

WASHINGTON, DC

The purpose of this study was to correlate the microbiology of serous otitis media in children with the duration of the condition and the patient's age. Aspirates of serous ear fluids from 114 children were examined for aerobic and anaerobic bacteria. Bacterial growth was noted in 47 patients (41%). Aerobic organisms only were recovered in 27 aspirates (57% of the culture-positive aspirates); anaerobic bacteria only in 7 (15%); and mixed aerobic and anaerobic bacteria in 13 (28%). A total of 83 bacterial isolates were recovered, accounting for 1.8 isolates per specimen (1.2 aerobes and 0.6 anaerobe). There were a total of 57 aerobic isolates, including *Haemophilus influenzae* (15 isolates), *Streptococcus pneumoniae* (13), and *Staphylococcus* sp (12). Twenty-six anaerobes were recovered, including anaerobic gram-positive cocci (10), *Prevotella* spp (8), and *Propionibacterium acnes* (4). The rate of positive cultures (20 of 36; 56%) was higher in patients younger than 2 years of age than in those older than 2 years of age (27 of 78; 35%). *Streptococcus pneumoniae* and *H influenzae* were more often isolated in children younger than 2 years of age and those with effusion for 3 to 5 months, whereas anaerobes were recovered more often in those older than 2 years of age and those with effusion for 6 to 13 months. These data illustrate the effects of the length of effusion and age on the recovery of aerobic and anaerobic bacteria in serous otitis media.

KEY WORDS — anaerobes, beta-lactamase, *Haemophilus influenzae*, serous otitis media, *Streptococcus pneumoniae*.

INTRODUCTION

Persistence of middle ear effusion (MEE) in the form of serous otitis media (SOM) occurs in as many as two thirds of children after an episode of acute otitis media (AOM) and takes as long as 3 months to resolve in 90% of patients. Bacteria can be recovered from 21% to 52% of specimens of these MEEs,[1-5] suggesting their potential role in the persistence of the condition. The bacteria recovered from the MEEs were similar to those isolated from AOM and include *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*.[1-5]

Most studies concerning the microbiology of SOM have not correlated the recovery of organisms with the patient's age or with the duration of the MEE after the occurrence of AOM. One recent report did, however, find a correlation between the microbiological findings and these factors.[6] However, methods for isolation of anaerobic bacteria were not used in that study.

This study reports our experience in the recovery of aerobic and anaerobic bacteria from MEEs of children with SOM and correlates the isolation of the organisms with the patient's age and the duration of MEE. This study includes data from our earlier publication.[7] However, additional patients are included in the analysis.

PATIENTS AND METHODS

One hundred fourteen children (62 male and 52 female) were studied. Their ages ranged from 14 months to 11 years (median age, 3.5 years). Forty-one of the children were younger than 2 years. The MEE specimens included were either serous or mucoid; no purulent specimens were included. After AOM or recurrent otitis media, all of the children underwent placement of tympanic ventilation tubes to correct persistent MEE of at least 3 months' duration. The patients' clinical data were obtained by a questionnaire completed by the parents and by review of the patients' records. Bilateral tympanocentesis was performed in 32 patients. Although 2 ear aspirates were obtained from these 32 patients, only 1 aspirate per patient (the one that contained more organisms) was considered in the final analysis. None had any signs or symptoms of acute infection or had undergone antimicrobial therapy for at least 2 weeks before sample collection; 54 had received no antimicrobial agents during the 3 months before sample collection. However, the patients had undergone many courses of antimicrobial therapy within the year before surgery. The agents used included amoxicillin, amoxicillin-clavulanate, penicillin V, cephalosporins,

---

From the School of Medicine, George Washington University (all authors), and the School of Medicine, Georgetown University (Brook), Washington, DC.

CORRESPONDENCE — Itzhak Brook, MD, MSc, PO Box 70412, Chevy Chase, MD 20813-0412.

TABLE 1. BACTERIA ISOLATED FROM 48 CULTURE-POSITIVE SEROUS EFFUSIONS

	No. of Isolates
Aerobic bacteria	
Streptococcus pneumoniae	13
Streptococcus pyogenes	2
Streptococcus group D	1
α-Hemolytic streptococci	4
γ-Hemolytic streptococci	3
Staphylococcus aureus	8 (8)
Staphylococcus epidermidis	4 (2)
Moraxella catarrhalis	6 (6)
Haemophilus influenzae	15 (7)
Escherichia coli	1 (1)
Subtotal	57 (24)
Anaerobic bacteria	
Peptostreptococcus spp	8
Streptococcus constellatus	2
Veillonella alcalescens	2
Propionibacterium acnes	4
Fusobacterium nucleatum	2 (1)
Prevotella melaninogenica	5 (2)
Prevotella intermedia	3 (1)
Subtotal	26 (4)
Total	83 (28)

Data in parentheses are numbers of β-lactamase–producing bacteria.

erythromycin, azithromycin dihydrate, clarithromycin, and trimethoprim-sulfamethoxazole.

The MEEs were collected in the operating room with the patient under general anesthesia. The external auditory canal was cleared of cerumen with a blunt curette. The canal was then filled with povidone-iodine solution for 3 minutes. Removal of this solution was accomplished by irrigation with 50 mL of sterile saline solution, and the excess saline solution was absorbed with sterile cotton. The external surface of the tympanic membrane was then swabbed with a sterile cotton applicator, which was immediately streaked on culture media and incubated for aerobic and anaerobic bacteria.

With a surgical microscope, a myringotomy incision was made in the tympanic membrane. Under direct visualization, the effusion was aspirated through sterile polyethylene tubing attached to a disposable middle ear fluid collector. After aspiration, excess air was evacuated from the collecting cup by filling the cup with prereduced thioglycolate broth. It was sealed and delivered to the microbiology laboratory, where the specimen was plated on aerobic and anaerobic media within 5 to 10 minutes of collection.

Sheep blood (5%), chocolate, and MacConkey agar plates were inoculated for the isolation of aerobic organisms. The plates were incubated at 35°C and examined at 24 and 48 hours. For the isolation of anaerobic bacteria, the specimens were plated to a prereduced vitamin K1–enriched *Brucella* blood agar, an anaerobic blood agar containing kanamycin and vancomycin, an anaerobic blood agar containing phenylethyl alcohol and an enriched thioglycolate broth.[8] All media were incubated in anaerobic jars and examined at 48 and 96 hours. The thioglycolate broth was incubated for 14 days. Aerobic bacteria and anaerobic bacteria were identified by conventional methods.[8,9] β-Lactamase activity was determined in all isolates by the chromogenic cephalosporin analog 87/312 method.[10] Statistical significance in the prevalence of recovery of bacteria was calculated by a χ^2 test with a Yates correction.

RESULTS

Bacterial growth occurred in 47 of the 114 patients (41%). Aerobic organisms alone were recovered in 27 aspirates (57% of the culture-positive aspirates), anaerobic bacteria alone in 7 (15%), and mixed aerobic and anaerobic bacteria in 13 (28%). Thus, aerobic bacteria were recovered in 85% and anaerobes in 43% of the aspirates with bacterial growth. No preoperative cultures of the external surface of the tympanic membrane showed bacterial growth.

A total of 83 bacterial isolates were recovered from the patients (Table 1), accounting for 1.8 isolates per specimen (1.2 aerobes and 0.6 anaerobe). Fifty-seven isolates were aerobic. The predominant isolates were *H influenzae* (15 isolates), *Staphylococcus aureus* (8), *S pneumoniae* (13), and *M catarrhalis* (6). A total of 26 anaerobic isolates were recovered. The predominant anaerobes were anaerobic gram-positive cocci (10 isolates), pigmented *Prevotella* spp (8), and *Propionibacterium acnes* (4). β-Lactamase production was observed in 28 isolates recovered from 19 patients (17%; Table 1).

The number of isolates per specimen ranged from 1 to 7. One isolate per specimen was recovered in 31 instances (66%). The organisms most frequently recovered as a single isolate were, in descending order of frequency, *H influenzae* (11 times), *S aureus* (6), *S pneumoniae* (5), and *P acnes* (2).

The rate of cultures with bacterial growth was higher in children younger than 2 years of age (20 of 36; 56%) than in those older than 2 years of age (27 of 78; 35%; $p < .05$). Correlation of culture results with age revealed a higher rate of recovery of *S pneumoniae* and *H influenzae* among those younger than 2 years of age ($p < .05$; Table 2). Conversely, anaerobic bacteria were isolated in greater numbers in patients older than 2 years of age ($p < .05$).

The proportion of isolation of *H influenzae* and *S pneumoniae* was also higher in the 26 children who had MEE for 3 to 5 months, as compared with the 21

TABLE 2. BACTERIA ISOLATED AND AGE OF CHILD

	Age of Child					
	<2 y (N = 20)		*2 to 13 y (N = 27)*		*Total (N = 47)*	
Bacteria Isolated	*No.*	*%*	*No.*	*%*	*No.*	*%*
S pneumoniae	9*	24	4	9	13	16
H influenzae	10*	26	5	11	15	18
M catarrhalis	3	8	3	7	6	7
Staphylococcus spp	5	13	7	16	12	14
Anaerobic bacteria	7*	18	19	42	26	31
Other bacteria	4	11	7	16	11	13
Total	38	100	45	100	83	100

*$p < .05$.

TABLE 3. BACTERIA ISOLATED AND PERSISTENCE OF MIDDLE EAR EFFUSION

	Persistence of Middle Ear Effusion					
	3 to 5 mo (N = 26)		*6 to 13 mo (N = 21)*		*Total (N = 47)*	
Bacteria Isolated	*No.*	*%*	*No.*	*%*	*No.*	*%*
S pneumoniae	11*	27.5	2	5	13	16
H influenzae	12*	30	3	7	15	18
M catarrhalis	4	10	2	5	6	7
Staphylococcus spp	3	7.5	9†	21	12	14
Anaerobic bacteria	7	17.5	19†	44	26	31
Other bacteria	3	7.5	8†	19	11	13
Total	40	100	43	100	83	100

*$p < .05$.
†$p < .001$.

children who had persistence of MEE for 6 to 13 months ($p < .05$; Table 3). *Staphylococcus* spp and anaerobic and other bacteria were more often isolated in those with MEE of 6 to 13 months' duration ($p < .001$).

DISCUSSION

This study illustrates the ability to recover aerobic and anaerobic bacteria in 41% of children with SOM. Anaerobic bacteria only were recovered in 7 of the culture-positive aspirates (15%), and in an additional 13 aspirates (28%), they were mixed with aerobic bacteria.

The anaerobes recovered from our patients were similar to those we previously recovered from children with AOM[11] and chronic otitis media[12] and are all known to be part of the oropharyngeal flora.[13] *Peptostreptococcus* spp were the predominant anaerobes we recovered in AOM,[11] and *Prevotella* spp predominated in chronic otitis media.[12]

That anaerobes were recovered more often from those who had MEE for longer than 3 months and those older than 2 years of age is in accord with their known predominance in chronic ear and other upper respiratory tract infections such as chronic sinusitis.[14] A transition in the microbial flora of the inflamed middle ear from aerobic to anaerobic bacteria was recently demonstrated[15] and may also take place in SOM.

Our ability to recover anaerobes in many of the aspirates may have been due to the meticulous methods used in the collection and transportation of the specimens. We were careful to avoid unnecessary suction while aspirating the ear fluid, thus minimizing exposure of the specimen to oxygen. In addition, we expelled all air from the collecting vial by adding prereduced thioglycolate broth before its transportation to the laboratory. Further, the time between collection and incubation of the culture in an anaerobic environment was less than 10 minutes. These measures have undoubtedly contributed to our ability to recover anaerobes.

The finding of *S pneumoniae* and *H influenzae* as the predominant isolates confirms previous studies of SOM.[1-6] The finding of high numbers of β-lactamase–producing isolates is not surprising, because all of the patients had undergone numerous courses of antimicrobial therapy for their ear infections.

Our finding of a correlation between the microbiological profile of MEE of SOM and the patient's age supports the results of Jero and Karma[6] and Healy and Teele,[3] who also showed that the proportion of recovery of *S pneumoniae* and *H influenzae* was higher in children younger than 2[6] or 3[3] years of age. However, Riding et al[16] did not find such a correlation.

The presence of pathogens often found in AOM in the ears of children with SOM that lasted 5 months or less, and of pathogens found in chronic otitis media in the ears of those with SOM that lasted 6 months or longer, may have implications for the management of SOM, if bacteria play a role in this condition. The effect of the patient's age and the duration of MEE on the microbiological profile of SOM may have practical implications if antimicrobial therapy is warranted. Further studies are needed to elucidate whether the antimicrobials used to treat SOM that does not resolve spontaneously should be adjusted to cover the specific organisms recovered from these patients.

ACKNOWLEDGMENT — The authors acknowledge the secretarial assistance of Sarah Blaisdell.

REFERENCES

1. Senturia BH, Gessert CF, Carr CD, Baumann ES. Studies concerned with tubotympanitis. Ann Otol Rhinol Laryngol 1958;67:440-67.
2. Bluestone CD, Stephenson JS, Martin LM. Ten-year re-

view of otitis media pathogens. Pediatr Infect Dis J 1992;11:7-11.

3. Healy GB, Teele DW. The microbiology of chronic middle ear effusions in children. Laryngoscope 1977;87:1472-8.
4. Giebink G, Mills L, Huff S, et al. The microbiology of serous and mucoid otitis media. Pediatrics 1979;63:915-9.
5. Sipila P, Jokipii MM, Jokipii L, et al. Bacteria in the middle ear and ear canal of patients with secondary otitis media and with non-inflamed ears. Acta Otolaryngol (Stockh) 1981;92:123-30.
6. Jero J, Karma P. Bacteriological findings and persistence of middle ear effusion in otitis media with effusion. Acta Otolaryngol Suppl (Stockh) 1997(suppl 529):22-6.
7. Brook I, Yocum P, Shah K, Feldman B, Epstein S. Aerobic and anaerobic bacteriologic features of serous otitis media in children. Am J Otolaryngol 1983;4:389-92.
8. Summanen P, Barron EJ, Citron DM, Strong CA, Wexler HM, Finegold SM. Wadsworth anaerobic bacteriology manual. 5th ed. Belmont, Calif: Star Publishing, 1993.
9. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 6th ed. Washington, DC: American Society for Microbiology, 1995.
10. O'Callaghan CH, Morris A, Kirby SM, Shingler AH. Novel method for detection of beta-lactamase by using a chromatogenic cephalosporin substrate. Antimicrob Agents Chemother 1972;1:283-8.
11. Brook I. Otitis media in children: a prospective study of aerobic and anaerobic bacteriology. Laryngoscope 1979;89:992-7.
12. Brook I, Finegold SM. Bacteriology of chronic otitis media. JAMA 1979;241:487-8.
13. Bowden GH, Hamilton IR. Survival of oral bacteria. Crit Rev Oral Biol Med 1998;9:54-85.
14. Brook I. Bacteriological features of chronic sinusitis in children. JAMA 1981;246:967-9.
15. Brook I. Microbial dynamics of persistent purulent otitis media in children. J Pediatr 1996;128:237-40.
16. Riding KH, Bluestone CD, Michaels RH, Cantekin EI, Doyle WJ, Poziviak CS. Microbiology of recurrent and chronic otitis media with effusion. J Pediatr 1978;93:739-43.

≈

EIGHTH SYMPOSIUM ON COCHLEAR IMPLANTS IN CHILDREN

The Eighth Symposium on Cochlear Implants in Children will be held in Los Angeles, California, February 28 to March 3, 2001. For information, visit the web site www.hei.org/ci2001; telephone Caroline Sakai at (213) 353-7001; or write to House Ear Institute/CI2001, 2100 W Third St, Los Angeles, CA 90057.

Ann Otol Rhinol Laryngol 110:2001

VOLUMETRIC AND DIMENSIONAL ANALYSIS OF THE GUINEA PIG INNER EAR

YUSUKE SHINOMORI, MD
DIANE D. JONES
DEBRA S. SPACK
ROBERT S. KIMURA, PHD

BOSTON, MASSACHUSETTS

The objective of this study was to provide accurate volumetric data on the fluid spaces and soft tissue in the guinea pig inner ear by measuring all histologic serial sections by means of Metamorph Imaging Software at 400× to 1,000× magnification. The total endolymph volume of the inner ear was 4.691 mm^3, of which 1.501 mm^3 was in the cochlea, 3.090 mm^3 in the vestibular labyrinth, and 0.100 mm^3 in the endolymphatic duct and sac. The total perilymph volume was 15.938 mm^3, of which 8.867 mm^3 was in the cochlea and 7.071 mm^3 in the vestibular labyrinth. The volume of the organ of Corti per millimeter length increased toward the apex, but the volumes of the stria vascularis, spiral ligament, and spiral limbus decreased. The volume of the macula utriculi was larger than that of the macula sacculi. The measurement of the luminal surface area of the stria vascularis was 3.944 mm^2, and that of the vestibular dark cells was 5.772 mm^2.

KEY WORDS — guinea pig, inner ear, volume.

INTRODUCTION

Although guinea pigs have been used extensively for otologic research, the inner ear volumes have not been documented in detail and need to be updated by means of a precise technique. Past morphometric studies examined the cochleas of the guinea pig,[1] cat, dog, and human[2,3] and the vestibular labyrinths of various species.[4-7] The most frequently used method was to measure every 10th section or every other section by tracing the images projected on white paper.[1,3] Another method was to withdraw inner ear fluid with a micropipette.[2] More recently, a magnetic resonance microscopy technique was introduced to measure the volume of inner ear fluids.[8,9] In all of these methods, various degrees of estimation were involved.

In order to obtain accurate volumetric data, we measured every serial section from the inner ears of 3 guinea pigs with the use of a computer. These measurements provide complete volume information on the endolymphatic and perilymphatic spaces and soft tissues. In addition, the luminal surface areas of the stria vascularis and vestibular dark cells were measured to compare the distributions of secretory cells in the cochlea and the vestibular labyrinth. These results provide data useful for comparative anatomic studies and for physiological and clinical research techniques such as the irrigation of drugs through the perilymphatic space, injection of tracer substances, withdrawal of fluids for biochemical analysis, and determination of in vitro enzyme activity of inner ear tissues.

MATERIALS AND METHODS

Two different groups of guinea pigs were used for different purposes. For measurement of the volumes, 3 normal left ears from Duncan Hartley albino guinea pigs weighing 316 to 346 g were used. For measurement of the luminal surface areas of the stria vascularis and vestibular dark cells, 3 normal left ears from pigmented guinea pigs weighing 260 to 300 g were used. In the pigmented animal ears, measurement of dark cell areas was easier, because they were underlined by melanocytes.[10] Temporal bones were fixed with Heidenhain-Susa fixative by intracardiac perfusion, and both ears were removed in one block with the midbrain intact. The bullae were opened and the specimens were decalcified in 5% trichloroacetic acid, dehydrated, and embedded in celloidin. For sectioning, both cochleas were oriented in a horizontal plane and cut symmetrically at 20-μm thickness starting at the promontory of the basal turn. The number of sections required to cut through the entire labyrinth in a horizontal plane was 285 to 288. All serial sections of inner ears from the nonpigmented ani-

---

From the Department of Otology and Laryngology, Harvard Medical School (Shinomori, Kimura), and the Department of Otolaryngology, Massachusetts Eye and Ear Infirmary (all authors), Boston, Massachusetts. Supported by National Institutes of Health/National Institute on Deafness and Other Communication Disorders grant RO1 DC00073. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Massachusetts Eye and Ear Infirmary (protocol approval #9006021).

CORRESPONDENCE — Robert S. Kimura, PhD, Dept of Otolaryngology, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114.

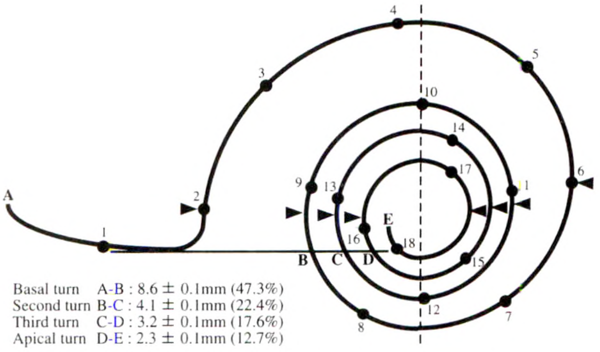

4
5
3
10
14
17
9
13
11
6
A
2
E
16
18
1
B
C
D
15
12
7
8
Basal turn A-B : 8.6 ± 0.1mm (47.3%)
Second turn B-C : 4.1 ± 0.1mm (22.4%)
Third turn C-D : 3.2 ± 0.1mm (17.6%)
Apical turn D-E : 2.3 ± 0.1mm (12.7%)

Fig 1. Schematic spiralgram based on 2-dimensional reconstruction method. Total cochlear length (18.17 mm) is divided into turns by solid line that is horizontal and tangential to hook portion. Total cochlear length is also divided into 9 segments by 8 points drawn on mid-semicircles of turns (arrowheads).

mals and every 10th section from the pigmented animals were stained with hematoxylin and eosin.

Cochlear spiralgrams were made from 3 left ears of albino guinea pigs with the 2-dimensional reconstruction method to obtain the volume of fluids and soft tissues in each cochlear turn and along the cochlear length. The mean cochlear length (±SD) determined at the apical junction of the inner and outer pillar cells was 18.17 ± 0.15 mm. The cochlear turns were determined by drawing a horizontal line that was tangential to the hook portion and intersected other cochlear turns on the spiralgram (Fig 1). The mean lengths of the basal, second, third, and apical turns were 8.6 ± 0.1, 4.1 ± 0.1, 3.2 ± 0.1, and 2.3 ± 0.1 mm, respectively. In order to calculate the volume per millimeter length, the total cochlear length was divided into 9 segments by determining 8 points, 1 on the mid-semicircle of each turn, so that radial cross sections of the scala media (SM) could be obtained (Fig 1). The distances between these points were identified on the spiralgram, and the volume of each segment was divided by its length. The mean distances of the 8 points on the mid-semicircles of the turns were 2.0, 6.0, 8.8, 11.1, 12.9, 14.6, 16.1, and 17.3 mm, from the basal end.

Each fluid compartment and soft tissue was traced on the video screen at 400× to 1,000× magnification with Metamorph Imaging Software (Universal Imaging Corp, West Chester, Pa). The volume was calculated by multiplying the sum of the areas by the thickness of each section. The luminal surfaces of the stria vascularis and vestibular dark cells were traced in the same manner, and the areas were calculated by multiplying the sum of the surface lengths of every 10th section by 10 times the thickness of each section. The following structures were defined and measured. Demarcations are shown in the cochlea (Fig 2) and vestibular labyrinth (Fig 3).

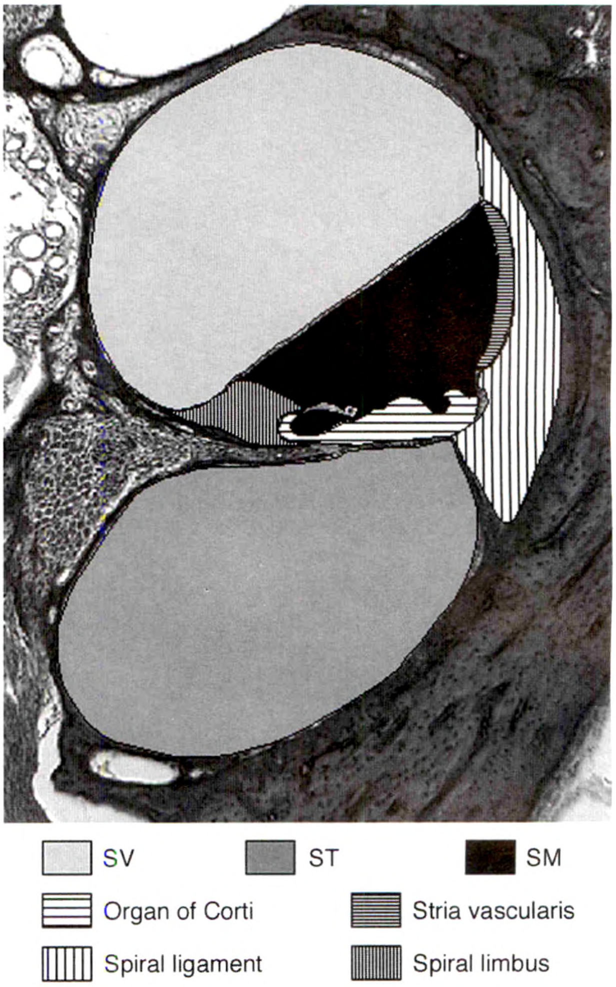


Fig 2. Basal turn of cochlea shows areas of measurements. SV — scala vestibuli, ST — scala tympani, SM — scala media.

Endolymph. We measured the endolymphatic space in the following locations.

1. The SM, excluding the tectorial membrane.

2. The saccule, including the ductus reuniens and saccular duct.

3. The utricle, including the utricular duct.

4. The superior ampulla (S ampulla), posterior ampulla (P ampulla), and lateral ampulla (L ampulla).

5. The superior semicircular canal (SSC) and posterior semicircular canal (PSC), including the common crus (CC), and the lateral semicircular canal (LSC).

6. The endolymphatic duct and sac (E duct and sac), including the endolymphatic sinus and the distal part of the endolymphatic sac.

Perilymph. We measured the perilymphatic space in the following locations.

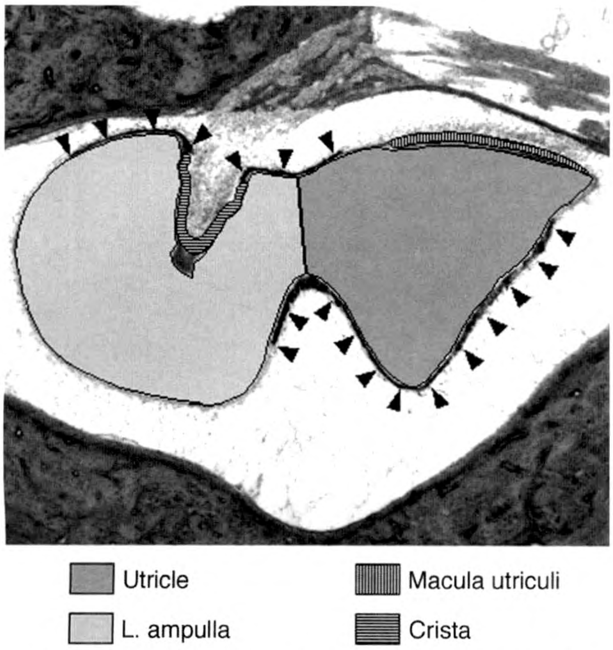


Fig 3. Vestibular labyrinth shows measurement areas of dark cells underlined by melanocytes (arrowheads), sensory-supporting epithelia, and arbitrary line drawn to indicate border of endolymphatic space between lateral ampulla (L. ampulla) and utricle.

1. The scala vestibuli (SV) has no clear landmark toward the vestibule. Therefore, the SV was defined as a perilymphatic area anterior to a line drawn from the posterior margin of the spiral ligament at the basal end to the anterior margin of the ductus reuniens or the saccule membrane. The area posterior to this line was considered the vestibular perilymphatic space.

2. The scala tympani (ST).

3. The cochlear aqueduct.

4. Vestibular perilymph was combined, because of the difficulty in determining the borderline of the fluid space. The measurements included the perilymphatic space of the saccule as defined above and the utricle, ampullae, canals, and loose connective tissues and nerve fibers below the sensory epithelia.

Soft Tissues. Composite volumes of different types of cells were measured, because these cells were considered to serve as a functional unit.

1. The organ of Corti, defined as sensory cells, its accessory cells (supporting cells, Claudius cells, Boettcher cells, and inner sulcus cells), and Corti's and Nuel's spaces.

2. The stria vascularis, with marginal, intermediate, and basal cells.

3. The spiral ligament, excluding the spiral prominence cells, the distinct part of the external sulcus cells, and the secondary osseous spiral lamina.

TABLE 1. ENDOLYMPH VOLUME

	Mean ± SD (mm³)
Cochlea	
Scala media	
Basal turn	0.746 ± 0.049
Second turn	0.363 ± 0.010
Third turn	0.253 ± 0.012
Apical turn	0.140 ± 0.009
Total	1.501 ± 0.071
Endolymphatic duct and sac	0.100 ± 0.029
Vestibular labyrinth	
Saccule	0.376 ± 0.039
Utricle + DR	0.858 ± 0.091
SSC + PSC + CC	0.667 ± 0.036
LSC	0.267 ± 0.009
Superior ampulla	0.312 ± 0.030
Posterior ampulla	0.326 ± 0.019
Lateral ampulla	0.284 ± 0.036
Total	3.090 ± 0.220
Total endolymph	4.691 ± 0.319

DR — ductus reuniens, SSC — superior semicircular canal, PSC — posterior semicircular canal, CC — common crus, LSC — lateral semicircular canal.

4. The spiral limbus, including the interdental cells.

5. The macula sacculi and macula utriculi, defined as sensory cells, supporting cells, and transitional cells.

6. The superior crista (S crista), posterior crista (P crista), and lateral crista (L crista), defined as sensory cells, supporting cells, transitional cells, and planum semilunatum.

7. The vestibular aqueduct, including the epithelial cells and connective tissue within the bony channel.

Luminal Surface Area of Secretory Cells. The luminal surface area, instead of the volume, was measured, because the height of the vestibular dark cells became very small and it was difficult to determine the precise volume.

1. The stria vascularis, defined as the luminal surface area of the marginal cells.

2. The vestibular dark cells, which are underlined by melanocyte layers in pigmented guinea pig ears.

RESULTS

The endolymph volume measurements of the SM, vestibular labyrinth, and E duct and sac are shown in Table 1. The mean endolymph volume was the largest in the SM, followed by the 3 ampullae, the utricle, and the saccule, in declining order (Fig 4). The total volume of the SM was approximately half that of the vestibular labyrinth. The mean endolymph

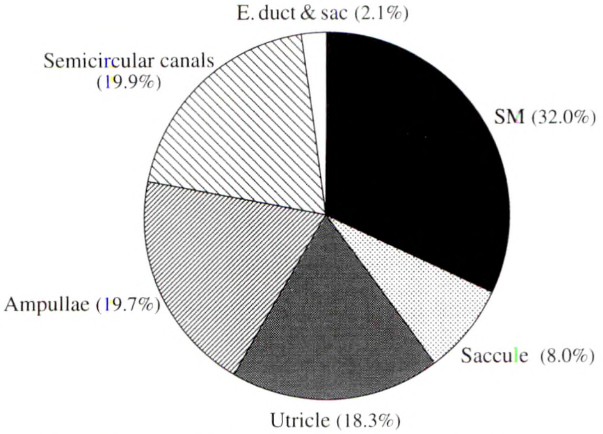


Fig 4. Ratio of different endolymphatic (E.) spaces.

volume of the SM was 1.501 mm³, and 69% of the volume was located at the basal turn. The endolymph volume of the SM per millimeter length increased from the basal end, reached its maximum at a site at about 20% of the cochlear distance, and remained the same up to 68% of the distance, at which point it gradually tapered toward the apex (Fig 5).

The endolymph volume of the utricle was 0.858 mm³, and that of the saccule was 0.376 mm³. The utricle endolymph volume was larger than that of the saccule (ratio, 2.28 to 1). The endolymph volumes of the ampullae were 0.312 mm³ for the S ampulla, 0.326 mm³ for the P ampulla, and 0.284 mm³ for the L ampulla. The volumes of the ampullae were similar, although the volume of the L ampulla was the smallest.

The perilymph volume measurements of the cochlea, vestibular labyrinth, and cochlear aqueduct are shown in Table 2. The total perilymph volume of the cochlea was 8.867 mm³, of which 4.211 mm³ was in the SV and 4.656 mm³ was in the ST. The volume of the SV varied, depending on the arbitrarily set borderline toward the cistern of the vestibule. If the borderline is extended to the membrana limitans to include the whole cistern of the vestibule, the SV volume is 7.405 mm³. Regarding the volume per millimeter length, the large SV volume at the basal turn decreased rapidly to a region at 20% of the cochlear distance from the basal end, and then tapered gradually toward the apex. The ST volume was very small at the hook region and increased to a peak at around 20% of the cochlear distance before it tapered and fell below the SV and SM volumes beyond the 75% distance (Fig 5). In the vestibular labyrinth, the total volume of the perilymphatic space was slightly smaller than that of the cochlear perilymph volume (ratio, 1 to 1.25).

The soft tissue volume measurements of the cochlea, vestibular labyrinth, and E duct and sac are shown in Table 3. The volume of the organ of Corti

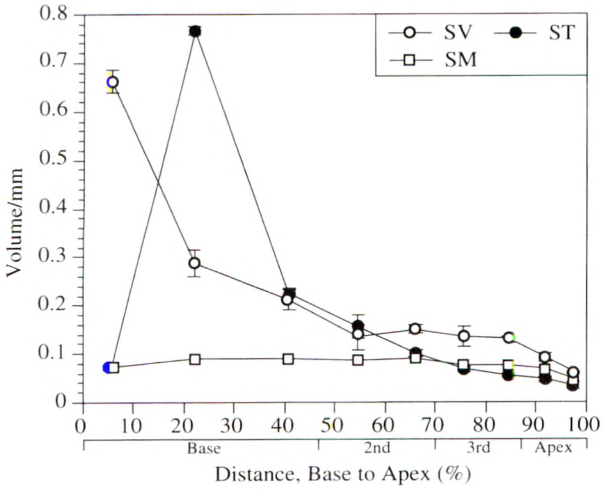


Fig 5. Volume of cochlear fluid compartments per millimeter length.

TABLE 2. PERILYMPH VOLUME

	Mean ± SD (mm³)
Cochlea	
Scala media	
Basal turn	2.988 ± 0.201
Second turn	0.590 ± 0.067
Third turn	0.436 ± 0.033
Apical turn	0.197 ± 0.014
Total	4.211 ± 0.272
Scala tympani	
Basal turn	3.794 ± 0.396
Second turn	0.554 ± 0.030
Third turn	0.208 ± 0.019
Apical turn	0.100 ± 0.009
Total	4.656 ± 0.452
Total	8.867 ± 0.626
Cochlear aqueduct	0.113 ± 0.032
Vestibular labyrinth	
Perilymph and subepithelial tissue	7.071 ± 0.153

TABLE 3. SOFT TISSUE VOLUME

	Mean ± SD (mm³)
Cochlea	
Spiral ligament	1.347 ± 0.138
Spiral limbus	0.185 ± 0.007
Organ of Corti	0.343 ± 0.007
Stria vascularis	0.134 ± 0.010
Vestibular labyrinth	
Macula sacculi	0.014 ± 0.001
Macula utriculi	0.024 ± 0.001
Superior crista	0.020 ± 0.002
Posterior crista	0.018 ± 0.003
Lateral crista	0.019 ± 0.003
Vestibular aqueduct*	0.185 ± 0.028

*Epithelia of endolymphatic duct and sac and connective tissue.

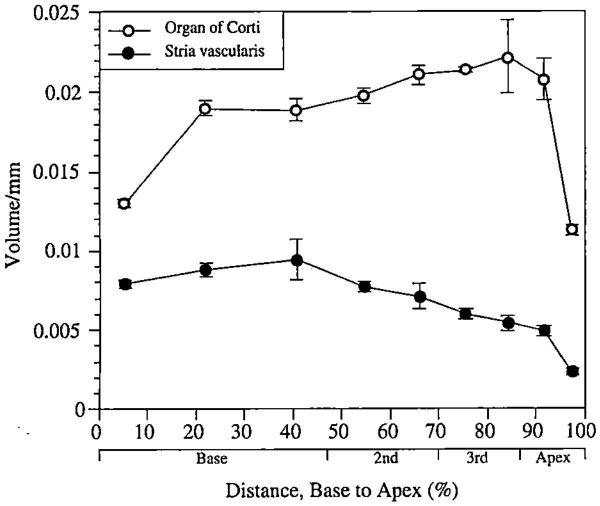


Fig 6. Volumes of organ of Corti and stria vascularis per millimeter cochlear length.

per millimeter length gradually increased from the basal end toward the second, third, and apical turns with fluctuation, and then rapidly tapered at the apical end (Fig 6). The largest volume was found at the third turn, about 85% of the cochlear distance from the basal end, and was 1.7 times larger than that of the basal turn. The volume of the stria vascularis per millimeter cochlear length was large at the basal turn, reaching the highest point at a site about 40% of the cochlear distance from the basal end and decreasing gradually toward the apex (Fig 6). The volume of the spiral ligament per millimeter length was largest at around 20% of the cochlear distance and then decreased sharply at about 55% of the distance toward the apex (Fig 7). The volume of the spiral limbus tapered off smoothly from the base to the apex (Fig 7).

The volume of the sensory epithelium and supporting cells of the macula utriculi was the largest and that of the macula sacculi was the smallest among the vestibular sense organs. The macula utriculi was 1.7 times larger than the macula sacculi. The volumes of the superior, lateral, and posterior canal cristae were all similar.

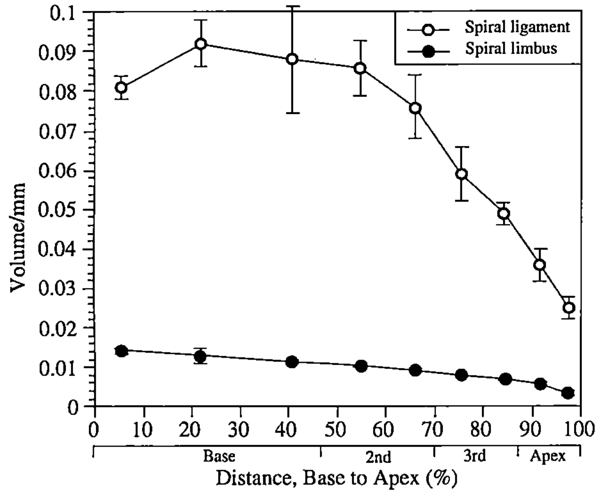


Fig 7. Volumes of spiral ligament and spiral limbus per millimeter cochlear length.

TABLE 4. SECRETORY CELL AREA

	Mean ± SD (mm²)
Cochlea	
Stria vascularis	
Basal turn	2.091 ± 0.194
Second turn	0.813 ± 0.094
Third turn	0.633 ± 0.098
Apical turn	0.407 ± 0.044
Total	3.944 ± 0.346
Vestibular labyrinth	
Utricle	3.527 ± 0.221
Superior ampulla	0.430 ± 0.037
Posterior ampulla	0.515 ± 0.052
Lateral ampulla	0.336 ± 0.103
Common crus	0.828 ± 0.195
LSC (nonampullated end)	0.136 ± 0.025
Total	5.772 ± 0.288

The luminal surface areas of the stria vascularis and vestibular dark cells are shown in Table 4. The surface areas of the stria vascularis were smaller than those of the vestibular dark cells, with a ratio of 1 to 1.46. In the vestibular labyrinth, the utricle showed the largest distribution of total vestibular dark cells (61.1%), and the L ampulla showed the smallest distribution (5.8%) among the ampullae. The CC and the nonampullated end of the LSC demonstrated a distribution of dark cells continuous with those of the utricle. The distribution of dark cells in the CC was larger than that in each crista. Only the saccule did not contain dark cells.

The volumes of the cochlear endolymph and perilymph were also estimated from each set of every 10th section, and they were compared with those from every section mentioned above (Table 5). The volumes of the SM from every 10th section (±SD) were 1.555 ± 0.056 mm^3, 1.526 ± 0.028 mm^3, and 1.421 ± 0.015 mm^3 in the first, second, and third specimens, respectively. The largest variation was found in the SM of the first specimen, with a 5.53% difference among section measurements. Similarly, in the SV and ST, the largest variations were 2.66% and 3.92%, respectively. These results suggested that variances of measurements between every 10th section and every section group were small.

DISCUSSION

The volume of the SM, 1.501 mm^3, was close to previously reported data from our laboratory.[11] The endolymph volume of the cochlea is 32.0% of the

TABLE 5. COCHLEAR ENDOLYMPH AND PERILYMPH VOLUME MEASURED IN ALL SECTIONS VERSUS EVERY TENTH SECTION

		Ten Sets of Every Tenth Section										
	All Sections	*1-280*	*2-281*	*3-282*	*4-283*	*5-284*	*6-285*	*7-286*	*8-287*	*9-288*	*10-279*	*SD*
Scala media												
1	1.555	1.469	1.582	1.587	1.598	1.632	1.588	1.570	1.534	1.478	1.495	0.056
2	1.526	1.531	1.549	1.559	1.550	1.531	1.476	1.491	1.496	1.517	1.524	0.028
3	1.421	1.443	1.434	1.418	1.407	1.413	1.399	1.406	1.433	1.436	1.420	0.015
Average	1.501	1.481	1.522	1.521	1.518	1.525	1.488	1.489	1.488	1.477	1.480	
Scala vestibuli												
1	4.170	4.059	4.263	4.207	4.231	4.126	4.091	4.140	4.180	4.200	4.200	0.064
2	4.501	4.501	4.592	4.584	4.536	4.416	4.431	4.423	4.468	4.455	4.506	0.066
3	3.961	4.020	4.005	3.871	3.889	3.960	3.955	3.981	4.001	3.974	3.958	0.038
Average	4.210	4.193	4.287	4.221	4.219	4.167	4.159	4.181	4.216	4.243	4.221	
Scala tympani												
1	4.520	4.343	4.544	4.532	4.506	4.587	4.551	4.560	4.540	4.554	4.486	0.068
2	5.191	5.140	5.226	5.223	5.187	5.187	6.162	5.150	5.201	5.218	5.215	0.031
3	4.286	4.322	4.295	4.274	4.265	4.281	4.246	4.317	4.302	4.286	4.270	0.026
Average	4.655	4.601	4.688	4.676	4.653	4.578	4.653	4.676	4.681	4.686	4.657	

All serial sections were numbered from inferior (1) to superior (285 through 288).

total volume of the inner ear endolymph. This ratio is considerably higher than the 20.2% in the human inner ear.[3] Most recently, DeMott et al[9] reported that the volumes of the cochlear fluid spaces in guinea pigs measured by 3-dimensional magnetic resonance microscopy were 3.89, 1.20, and 4.76 mm^3 in the SV, SM, and ST, respectively. Their measurements of SV and SM volumes were considerably smaller than our data, but the ST volume was close. As far as the SV is concerned, most of these differences seemed to be due to the definition of the borderline between the SV and the cistern of the vestibule. However, the difference in the SM was presumed to be due to technical differences, such as the resolution of the images and specimen preparation. The normal volumes of endolymph and perilymph are the reference points for the amounts of substances to be injected into or fluid to be aspirated from the SM, endolymphatic sac, or perilymphatic space. If the injected or aspirated amount is too large, the results of such studies become complicated or even unreliable.

The sizes of the fluid compartments along the cochlear length from cross sections of the guinea pig cochlea were reported by Fernandez[1] as early as 1952. The volumes of the SM per millimeter of cochlear length in our present study were not as uniform as those of Fernandez's report, but corresponded roughly to the data reported by Salt et al.[8] The perilymph volumes of the SV and ST per millimeter length (Fig 5) did not correspond precisely to those of previous reports.[8] The peak of the ST in our study actually should be shifted to the basal side. The reason for this difference is that the cochleas were cut only in a horizontal plane, and the length of each segment at the basal turn was much greater than that of other turns.

The endolymph volumes of the saccule and utricle were larger than the previously reported data of 0.302 and 0.661 mm^3, respectively, in guinea pigs,[7] although the ratios are quite close. Our measurements showed that the endolymph volume of the L ampulla was the smallest, and that of the P ampulla, the largest. Curthoys et al[12] reported that no differences were found among the dimensions of the ampullae of the 3 canals in guinea pigs. This difference might be due to the variability in determining the borderline between the utricle and ampullae.

The auditory organ, including the organ of Corti, basilar membrane, and tectorial membrane, is known to demonstrate differences in size along its cochlear length. Past morphometric studies revealed that the width of the basilar membrane is greater in the apex than in the base.[1,13,14] Bohne and Carr[15] demonstrated that the cross-sectional area of the chinchilla organ of Corti gradually increased along the distance from the base to the apex. In this study, the volume of the organ of Corti was about 1.7 times greater at the apical turn than at the basal turn. These volume increases could be related to the width increase of the basilar membrane.

All other tissues decreased in size toward the cochlear apex. The volume of the stria vascularis at the apical turn was almost half that of the basal turn. The spiral ligament volume was about one third that of the basal turn. A decrease in size of the stria vascularis and an increase in size of the organ of Corti may have a profound impact on cochlear sensory cells when the stria vascularis, which supplies oxygen to this organ, becomes atrophic at the apical region. We see concomitant degeneration of sensory cells and strial atrophy in the apical turns of chronically hydropic cochleas.[16] In the vestibular labyrinth, the macula utriculi is significantly larger (1.7×) than the macula sacculi. This difference in size suggests that the sensory inputs to the brain from the macula utriculi play a dominant role in maintaining the body equilibrium.

The surface area of the strial marginal cells and vestibular dark cells plays an important role in transport of various ions into and out of the endolymph compartment. These cells are believed to secrete potassium ions into the endolymph. The luminal surface area of the vestibular dark cells is 1.46 times larger than that of the strial marginal cells, while the endolymph volume of the vestibular labyrinth is about 2.06 times larger than that of the cochlear endolymph. The endolymph volume in per-unit area of the secretory cells in the vestibular labyrinth is slightly larger (1.4×) than that in the cochlea. Hence, the vestibular dark cells supply endolymph in a larger volume, while the strial marginal cells supply a smaller volume, although the difference is small. If the secretory activities of the strial marginal cells and vestibular dark cells were similar, the potassium ion concentration in the endolymph of the vestibular labyrinth would be lower than that of the cochlea, or, conversely, the potassium ion concentration in the cochlear endolymph would be higher than that of the vestibular labyrinth.[17]

Tissue shrinkage is, more or less, an unavoidable problem when tissue is fixed for histologic examination. However, it is still controversial how much shrinkage is caused by fixatives. Brunschwig and Salt[18] reported that Heidenhain-Susa fixative causes 15.1% shrinkage and that 7.4% formaldehyde causes 5.4% shrinkage of the perimeter of the isolated Reissner's membrane. On the other hand, Igarashi et al,[5] in their study of human temporal bones that were processed for routine histologic examination, could not find any statistically significant difference in the volume of endolymph between Heidenhain-Susa–fixed and 10% formalin–fixed human temporal bones. They also reported that the semicircular canal duct of squirrel monkeys fixed in 10% formalin had 9.2% to 9.3% circumference shrinkage compared with fresh specimens.[6]

From the celloidin sections in our laboratory, we found that the volumes of the SM and the saccule of guinea pig ears fixed with Heidenhain-Susa fixative were 12.2% and 14.1% larger, respectively, than those of ears fixed with 10% neutral formalin. Reissner's membrane remained in a straight line with Heidenhain-Susa fixation, in contrast to the slight collapse shown with 10% formalin fixation. If Heidenhain-Susa fixation causes more tissue shrinkage than 10% formalin fixation, the cochlear volume should be larger and the saccule volume should be smaller than those of 10% formalin fixation. However, the saccule volume was larger. These observations suggest that the volume of the fluid compartments is not influenced by shrinkage alone, but perhaps also by the stiffness of the membrane induced by the fixative. We noticed that the neck is stiffer and the brain is firmer in animals perfused intracardially with Heidenhain-Susa fixative than in those fixed with 10% formalin. The position of stiff membranes is less likely to change than that of flexible membranes when viscous embedding medium abuts a membranous compartment.

Measuring every section of our guinea pig inner ears provided very accurate volumetric information. However, it required enormous time, effort, and technical skill. Since there were no major differences between the results of measuring every 10th section and those of measuring every section, it seems to be more practical to measure every 10th section.

REFERENCES

1. Fernandez C. Dimensions of the cochlea (guinea pig). J Acoust Soc Am 1952;24:519-23.

2. Maggio E. The humoral system of the labyrinth. Acta Otolaryngol Suppl (Stockh) 1966(suppl 218):77-135.

3. Igarashi M, Ohashi K, Ishii M. Morphometric comparison of endolymphatic and perilymphatic spaces in human temporal bones. Acta Otolaryngol (Stockh) 1986;101:161-4.

4. Fernandez C, Valentinuzzi M. A study on the biophysical characteristics of the cat labyrinth. Acta Otolaryngol (Stockh) 1968;65:293-310.

5. Igarashi M, O-Uchi T, Isago H, Wright WK. Utricular and saccular volumetry in human temporal bones. Acta Otolaryngol (Stockh) 1983;95:75-80.

6. Igarashi M, O-Uchi T, Alford BR. Volumetric and dimensional measurements of vestibular structures in the squirrel monkey. Acta Otolaryngol (Stockh) 1981;91:437-44.

7. Igarashi M, Isago H, O-Uchi T. Comparative morphometry of mammalian otolith organs. Ann Otol Rhinol Laryngol Suppl 1984;93(suppl 112):49-51.

8. Salt AN, Henson MM, Gewalt SL, Keating AW, DeMott JE, Henson OW Jr. Detection and quantification of endolymphatic hydrops in the guinea pig cochlea by magnetic resonance microscopy. Hear Res 1995;88:79-86.

9. DeMott JE, Thorne M, Salt AN, Henson MM, Henson OW Jr, Gewalt SL. Cochlear fluid space dimensions for six species derived from 3-D magnetic resonance images. ARO Abstracts 1999;22:208.

10. Kimura RS. Distribution, structure, and function of dark cells in the vestibular labyrinth. Ann Otol Rhinol Laryngol 1969; 78:542-61.

11. Kimura RS, Hutta J. Inhibition of experimentally induced endolymphatic hydrops by middle ear ventilation. Eur Arch Otorhinolaryngol 1997;254:213-21.

12. Curthoys IS, Markham CH, Curthoys EJ. Semicircular duct and ampulla dimensions in cat, guinea pig and man. J Morphol 1975;151:17-34.

13. Igarashi M, Mahon RG, Konishi S. Comparative measurements of cochlear apparatus. J Speech Hear Res 1968;11: 229-35.

14. Wada H, Sugawara M, Kobayashi T, Hozawa K, Takasaka T. Measurement of guinea pig basilar membrane using computer-aided three-dimensional reconstruction system. Hear Res 1998;120:1-6.

15. Bohne AB, Carr CD. Location of structurally similar areas in chinchilla cochleas of different lengths. J Acoust Soc Am 1979;66:411-4.

16. Kimura RS. Experimental blockage of the endolymphatic duct and sac and its effect on the inner ear of the guinea pig. A study on endolymphatic hydrops. Ann Otol Rhinol Laryngol 1967;76:664-87.

17. Ferrary E, Sterkers O. Mechanism of endolymph secretion. Kidney Int 1998;53(suppl 65):S98-S103.

18. Brunschwig AS, Salt AN. Fixation-induced shrinkage of Reissner's membrane and its potential influence on the assessment of endolymph volume. Hear Res 1997;114:62-8.

≈

SIXTH ASIAN RESEARCH SYMPOSIUM IN RHINOLOGY

The Sixth Asian Research Symposium in Rhinology will be held March 10-11, 2001, in Hong Kong, China. For information, contact 6th ARSR Secretariat, Department of Surgery, University of Hong Kong Medical Centre, Queen Mary Hospital, Pokfulam, Hong Kong SAR, China; telephone: (852) 2818 0232 or (852) 2855 4235; fax: (852) 2818 1186; e-mail: mededcon@hku.hk.

Ann Otol Rhinol Laryngol 110:2001

IMAGING CASE STUDY OF THE MONTH

ECTOPIC GROWTH HORMONE–SECRETING PITUITARY ADENOMA IN THE SPHENOID SINUS

DINO MADONNA, MD
ADY KENDLER, MD, PHD
AHMED M. S. SOLIMAN, MD
PHILADELPHIA, PENNSYLVANIA

INTRODUCTION

Pituitary gland involvement of the sinonasal tract is unusual. It is usually a result of downward extension of an intracranial tumor or, less commonly, a result of a tumor arising from ectopic remnants. We present a case of an ectopic growth hormone–producing pituitary adenoma of the sphenoid sinus.

CASE REPORT

A 60-year-old woman presented with complaints of headache, progressive facial pressure, and congestion that had lasted for 6 months. Her medical history was significant for a mixed papillary and Hürthle cell thyroid carcinoma for which she had undergone total thyroidectomy 6 months earlier.

Physical examination revealed coarse facial features; the remainder of her otolaryngological examination findings were normal. The findings of diagnostic nasal endoscopy were unremarkable. After an unsuccessful trial of medical therapy, a computed tomography scan of the paranasal sinuses was performed; it demonstrated a large, expansile mass of the sphenoid sinus (Fig 1). Magnetic resonance imaging revealed a large, inhomogeneous, nonenhancing mass, distinct from a normal pituitary gland, and involvement of the cavernous sinus (Fig 2). Laboratory evaluation revealed a normal serum growth hormone level. Transnasal endoscopic sphenoidotomy demonstrated a soft, rubbery mass within the sphenoid sinus; a biopsy was performed. Hematoxylin and eosin staining revealed large, ovoid cells with eosinophilic granular cytoplasm and round to ovoid nuclei (Fig 3). The results of immunohistochemical staining for growth hormone were positive and are demonstrated in Fig 4. The tumor was also positive for thyroid-stimulating hormone. Electron microscopy revealed abundant neurosecretory granules.

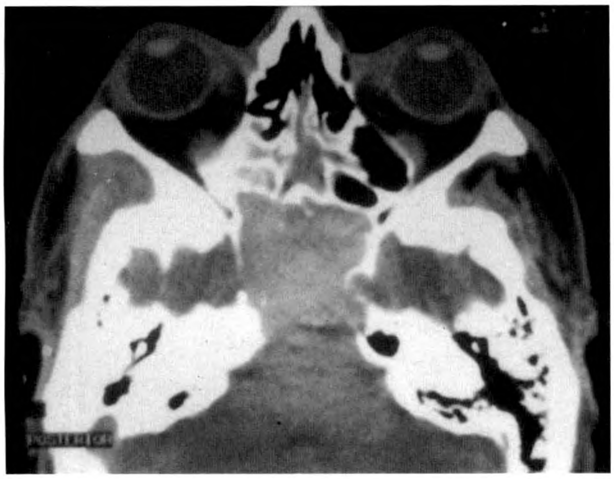


Fig 1. Unenhanced axial computed tomogram through sphenoid sinus shows large, expansile mass within sphenoid, eroding sella turcica.

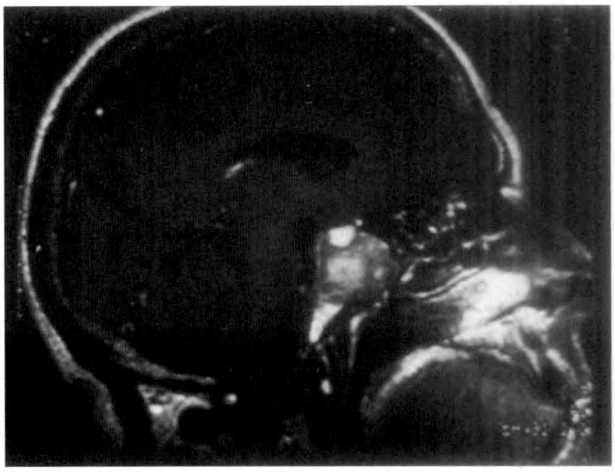


Fig 2. Gadolinium-enhanced sagittal magnetic resonance image through sphenoid sinus and sella turcica demonstrates large, inhomogeneous, nonenhancing mass distinct from normal pituitary gland, with involvement of cavernous sinus and destruction of floor of sella.

From the Departments of Otorhinolaryngology and Bronchoesophagology (Madonna, Soliman) and Pathology (Kendler), Temple University School of Medicine, Philadelphia, Pennsylvania.

CORRESPONDENCE — Ahmed M. S. Soliman, MD, Dept of Otorhinolaryngology and Bronchoesophagology, Temple University School of Medicine, 3401 N Broad St, Kresge West Bldg, Philadelphia, PA 19140.

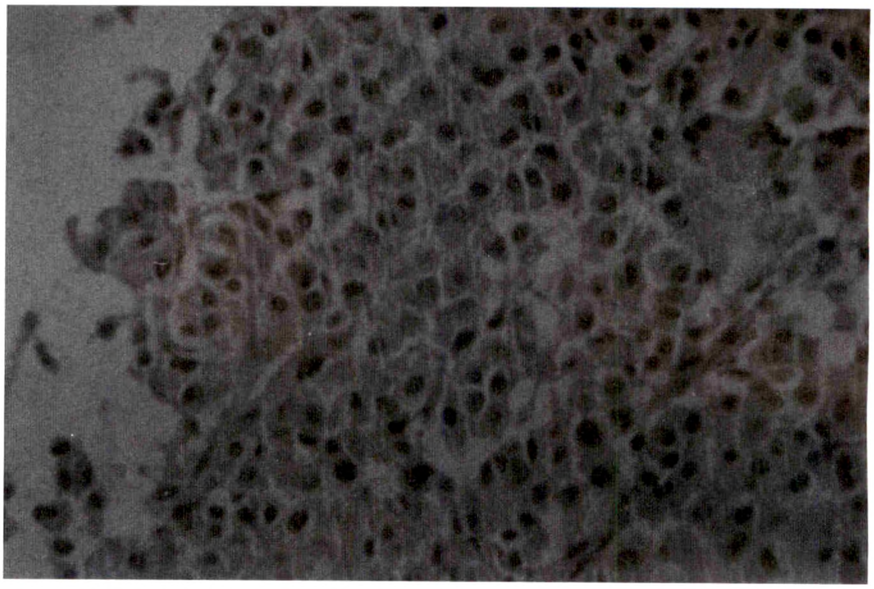


Fig 3. Large, ovoid cells with eosinophilic granular cytoplasm and round to ovoid nuclei (H & E, original ×100).

DISCUSSION

Ectopic pituitary adenomas (EPAs) are accessory masses of anterior pituitary tissue that have no connection to the intrasellar pituitary gland. These are uncommon lesions, with only 35 cases reported in the literature to date.[1] Both suprasellar and infrasellar sites have been described. The sphenoid bone and sinus represent the most common sites of EPA; other sites include the nasal cavity, nasopharynx, clivus, and temporal bone. Women are affected twice as often as men. The mean age at diagnosis is 49 years.[2]

Ectopic pituitary adenomas arise from ectopic rests of pituitary during development. During the fifth week of gestation, the oral ectoderm of Rathke's pouch comes into contact with the neuroectoderm making up the infundibulum to form the pituitary gland. The connection between Rathke's pouch and the oral cavity usually disappears by the sixth gestational week.[3] An ectopic pharyngeal hypophysis is found frequently in the mucoperiosteum of the vomerosphenoidal articulation at autopsy; remnants may also persist along the track of cephalic migration of Rathke's pouch and give rise to infrasellar ectopic pituitary tissue. Suprasellar ectopic sites may stem from anterior pituitary cells attached to the supradiaphragmatic portion of the pituitary stalk or from cells of the pars tuberalis.[2,4]

Clinical manifestations of an extracranial EPA may result from the lesion's space-occupying effects, destructive behavior, or hormonal activity. These include headache, nasal obstruction, chronic rhinosinusitis, visual field deficits, and cerebrospinal fluid leak. Fifty-eight percent of patients have evidence of hyperfunctional hormonal activity, including Cushing's

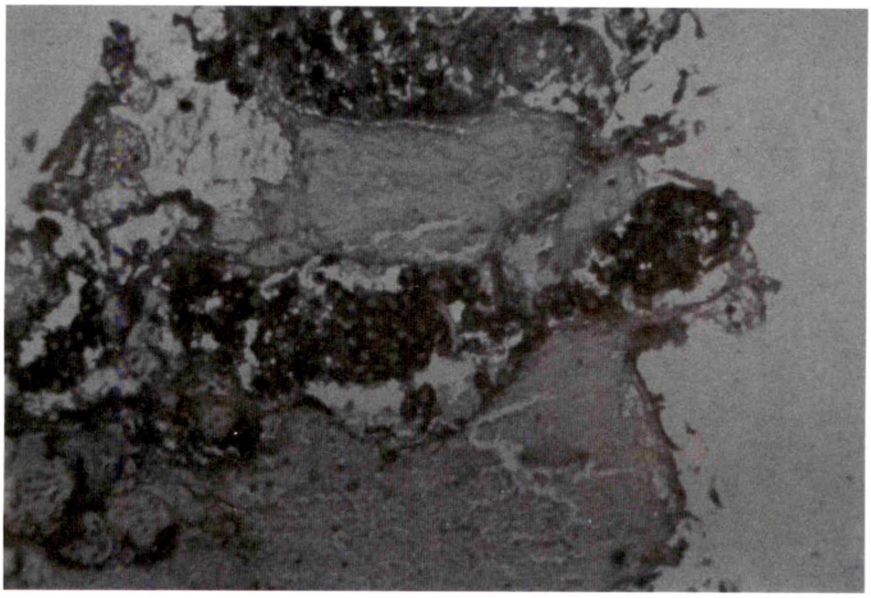


Fig 4. Tissue immunohistochemically stained for human growth hormone (original ×40). Note dark brown staining of cells.

syndrome, acromegaly, and hyperparathyroidism, at the time of presentation.[2] The extent of tumor and erosion of bone do not completely correlate with the severity of signs and symptoms.

Laboratory abnormalities may include elevated levels of any of the 6 pituitary hormones, which include luteinizing hormone, adrenocorticotropic hormone, prolactin, follicle-stimulating hormone, growth hormone, and thyroid-stimulating hormone. Because the amount of circulating hormone can fluctuate, serum levels may be normal at any one particular moment. Somatomedins may more reliably reflect acromegalic activity than serum growth hormone levels.[5]

Ectopic pituitary adenomas must be differentiated from other neuroendocrine tumors and from upper respiratory tract carcinomas such as adenocarcinoma, sinonasal undifferentiated carcinoma, and nasopharyngeal carcinoma.[1] Clival tumors, including chordoma, lymphoma, chondrosarcoma, plasmacytoma, giant cell tumor, and meningioma, must also be excluded. Intrasellar pituitary adenomas with sphenoid sinus invasion must also be considered. Radiographic images that demonstrate a normal intrasellar pituitary gland rule out the latter, but erosion of the sella turcica does not exclude an ectopic origin. Biopsy is necessary for histologic diagnosis.

Typical histologic features include cellular monotony and a delicate capillary network. By hematoxylin and eosin staining, the majority of EPAs are chromophobes.[6]

Immunohistochemical techniques that use specific pituitary hormone antibodies allow for a more precise diagnosis. Prolactin-producing tumors constitute one third of all pituitary tumors.[7] Growth hormone–producing adenomas make up 20% to 25% of all pituitary adenomas, with plurihormonal tumors being the principal subtype. Many of the acromegaly-associated adenomas are plurihormonal.[2,8] Despite immunohistochemical confirmation of multiple hormones, some of them may not be manifested clinically. Possible explanations include insufficient hormone levels, hormone production in a nonfunctional form, or inability to secrete.[8] Electron microscopy reveals typical secretory granules.

As with many endocrine neoplasms, the distinction between pituitary adenoma and carcinoma is difficult to make on histologic examination. Tumors with numerous mitoses and nuclear anaplasia may be indolent, and benign-appearing adenomas may be locally invasive and rarely metastasize. Recent studies have demonstrated increased levels of the specific tumor markers interleukin-6 and heat shock protein 27 in invasive pituitary adenomas as opposed to noninvasive ones.[9]

Optimal treatment consists of complete microsurgical excision. If total resection is unattainable, adjunctive radiotherapy is recommended. Bromocriptine mesylate, a dopamine agonist, has been effective in controlling prolactin-secreting tumors and reducing the mitotic rates of other pituitary adenoma types.[1,2] The somatomedin analog octreotide has been used as an adjunctive therapy for growth hormone–secreting adenomas and has resulted in normalization of growth hormone levels in 30% to 45% of patients.[10]

REFERENCES

1. Luk IS, Chan JK, Chow SM, Leung S. Pituitary adenoma presenting as sinonasal tumor: pitfalls in diagnosis. Hum Pathol 1996;27:605-9.
2. Langford L, Batsakis JG. Pituitary gland involvement of the sinonasal tract. Ann Otol Rhinol Laryngol 1995;104:167-9.
3. Moore KL. Before we are born: basic embryology and birth defects. 3rd ed. Philadelphia, Pa: WB Saunders, 1989:260-2.
4. Takahata T, Katayama Y, Tsubokawa T, Oshima H, Yoshino A. Ectopic pituitary adenoma occurring in the interpeduncular cistern. J Neurosurg 1995;83:1092-4.
5. Rieu M, Girard F, Bricaire H, Binoux M. The importance of insulin-like growth factor (somatomedin) measurements in the diagnosis and surveillance of acromegaly. J Clin Endocrinol Metab 1982;55:147-53.
6. Scheithauer BW, Koracs KT, Laws ER, Randall RV. Pathology of invasive pituitary tumors with special reference to functional classification. J Neurosurg 1986;65:733-44.
7. Burger PC, Scheithauer BW, Vogel FS. Surgical pathology of the nervous system and its coverings. 3rd ed. New York, NY: Churchill Livingstone, 1991:505.
8. Cooper DS, Wenig BM. Hyperthyroidism caused by an ectopic TSH-secreting pituitary tumor. Thyroid 1996;6:337-43.
9. Gandour-Edwards R, Kapadia SB, Janecka IP, Martinez AJ, Barnes L. Biological markers of invasive pituitary adenomas involving the sphenoid sinus. Mod Pathol 1995;8:160-4.
10. Davis DH, Laws ER, Ilstrup DM, et al. Results of surgical treatment for growth hormone–secreting pituitary adenomas. J Neurosurg 1993;79:70-5.

LETTER TO THE EDITOR

To the Editor:

We would like to comment on the recent Clinical Note in the *Annals of Otology, Rhinology & Laryngology* (Ress BD, Gross EM. Irreversible sensorineural hearing loss as a result of azithromycin ototoxicity. A case report. Ann Otol Rhinol Laryngol 2000;109:435-7). We also recently treated 2 patients who developed sudden hearing loss during the use of low-dose azithromycin. However, we were luckier than the authors, as the hearing returned to normal levels.

The first patient, 54 years old, had common influenza and had started to use azithromycin 500 mg daily for 3 days, although it was totally unnecessary. Unfortunately, azithromycin is an over-the-counter drug in Turkey, and patients have easy access to this medication without a prescription, although the insurance companies do not reimburse them when the drug is used in this way. The patient's hearing threshold in her left ear fell to 70 dB on the third day of the therapy. She was immediately put on high-dose corticosteroid and antiviral therapy, and her hearing threshold returned to the 30-dB range after 5 days. The second patient was a 60-year-old woman who had acute otitis media in addition to a possible viral upper respiratory tract infection. She was placed on a 5-day course of azithromycin (500 mg on day 1, followed by 250 mg daily for 4 days). She returned to the office complaining of ringing in the infected ear. On physical examination, the appearance of the eardrum was better, although there was a mild effusion. However, audiological testing revealed a mild-to-moderate sensorineural hearing loss with an average 10-dB air-bone gap in that ear. Her hearing also returned to normal after therapy.

We know that azithromycin may cause hearing loss when it is used long-term at high doses. We assume that an accompanying viral infection might lower the susceptibility threshold of patients for development of hearing loss while they are using low-dose azithromycin. This phenomenon might be similar to the development of a skin rash due to the use of amoxicillin during infectious mononucleosis.

In the light of this experience, we would like to thank the authors for their fine article and would like to recommend that azithromycin not be used as the first choice for upper respiratory tract infections, including otitis media, in adults or children, especially if there is the possibility of an additional viral infection.

BULENT MAMIKOGLU, MD
OSMAN MAMIKOGLU, MD
Ataturk Bul. 91/11
Kizilay, Ankara
Turkey

BOOK REVIEW

Pediatric Otolaryngology: Principles and Practice Pathways

Ralph Wetmore, Harland R. Muntz, and Trevor J. McGill, editors. Hard cover, illustrated, indexed, 1,042 pages, 2000. Thieme, New York, NY, $169.

Purpose: To encourage a thoughtful approach to the otolaryngological care of infants and children, with the knowledge that each child must be considered as an individual with specific needs. It is not meant to be an exhaustive reference. The format includes practice pathways and summary boxes that distill the major forms of care.

Contents: The text is divided into 6 parts. The initial part, Reviewing the General Care of the Pediatric Patient, presents the basics of molecular genetics with topics relevant to the pediatric population, such as anesthesia, vascular anomalies, soft tissue sarcomas, and psychological considerations. There are at least 10 chapters included in every section.

Attributes and Limitations: The volume contains finely crafted presentations of clinical information of important prognostic and management significance. The editors believe that the textbook is timely in that it arrives at the dawn of the new millennium and at the threshold of a molecular era of medicine. I say that it is timely because changes in modern medical care and workup of patient complaints have been streamlined through a number of mechanisms that ultimately have resulted in less access to medical resources and expertise. The information herein is extensive and informative. It does not legislate practical guidelines, but rather, encourages a thoughtful approach to the care of patients. As such, the text is a ready reference or a springboard for further investigation of a topic. In essence, the editors have fulfilled and accomplished their goals. A few areas could use improvement. A greater discussion of the role of food allergies in pediatric otolaryngology is needed. Page 231 states that otosclerosis in children is seen only in patients with osteogenesis imperfecta. On page 345, why give such an extensive discussion on congenital toxoplasmosis (and an excellent one, at that), when it is not a cause of sensorineural hearing loss? More information would be desirable on such subjects as midline lethal granulomatosis, aberrant carotid artery in the ear, and Tornwaldt's bursa and its differential diagnosis.

Applicability: This book is intended for health care professionals in training and those in clinical practice interested in diagnostic information on otolaryngology–head and neck conditions, whether in children or adults. As such (diagnostic information), it is near-encyclopedic in scope.

DENNIS G. PAPPAS, MD
Birmingham, Alabama

ANNALS

OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

FREE ONLINE ACCESS

Bringing you the most relevant developments and breakthroughs in otolaryngology—in time for you to put them into practice.

For the first time the *ANNALS* online is FREE to subscribers all over the world. Read it *before* you receive your journal!

http://www.annals.com

YES! Enter my subscription for 12 monthly issues of *ANNALS* for the calendar year January-December 2001. *(Please print.)*

☐ New ☐ Renewal

Name ______________________ E-mail ______________________

Address ______________________

City ______________ State ______________ ZIP ______________

Country ______________________ Specialty ______________________

2001 Annual Subscription Rates (includes online subscription)	USA	International Surface Mail (All Regions)
Institution	$240.00	$265.00
Individual	$170.00	$195.00
Resident/Student	$112.00	$129.00

☐ Payment enclosed* ☐ Visa ☐ Mastercard ☐ American Express

Card No. ______________________ Expiration Date ______________

Signature ______________________ Print name as it appears on card ______________________

*Checks must be in US dollars and drawn on a US bank payable to Annals Publishing Company.

Fax to (314) 367-4988 or mail to ANNALS PUBLISHING COMPANY, 4507 LACLEDE AVENUE, ST LOUIS, MO 63108, USA
E-mail: Manager@Annals.com • http://www.annals.com

11/00 Copyright ©2000 by Daiichi Pharmaceutical Corporation. All rights reserved.

Please see our full ad on pages 24a-d.

VOLUME 110 (2) — FEBRUARY 2001

— Founded in 1892 —

ANNALS

OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

Official Organ of the
AMERICAN LARYNGOLOGICAL ASSOCIATION
AMERICAN BRONCHO-ESOPHAGOLOGICAL ASSOCIATI

TWENTY-YEAR REPORT OF THE FIRST AUDITORY BRAIN STEM NUCLEUS IMPLANT

EARING IMPROVEMENT AFTER THERAPY FOR HYPERLIPIDEMIA IN PATIENTS WITH CHRONIC-PHASE SUDDEN DEAFNESS

EFFECTS OF EXPOSING THE OPENED ENDOLYMPHATIC SAC TO LARGE DOSES OF STEROIDS TO TREAT INTRACTABLE MENIERE'S DISEASE

POLY(ADENOSINE DIPHOSPHATE–RIBOSE) YNTHETASE INHIBITOR 3-AMINOBENZAMIDE ALLEVIATES COCHLEAR DYSFUNCTION INDUCED BY TRANSIENT ISCHEMIA

LONG-TERM STUDY OF ENDONASAL RAPLASTY AND REVIEW OF THE LITERATURE

RETROPHARYNGEAL AUTOLOGOUS FAT RANSPLANTATION FOR CONGENITAL SHORT PALATE: A NASOMETRIC ASSESSMENT OF FUNCTIONAL RESULTS

UNIVERSITY
LIBRARY

ANNALS
PUBLISHING CO.

WEB SITE—http://www.Annals.com

Adjust Your Expectations

WEERDA Distending Operating Laryngoscope

Make a well-adjusted choice in laryngeal surgery with the WEERDA Distending Operating Laryngoscope, now featuring a support frame on only one side to ensure easy instrument access. Dual turnscrews allow either proximal or distal expansion for rapid adjustments to the changing requirements of laryngeal surgery. For easy insertion, it narrows down to the size of a closed laryngoscope. Another simple adjustment and it opens wide to provide an improved working area.

But the WEERDA Distending Operating Laryngoscope is accommodating in so many other ways. A funnel-shaped widening at the entrance and broad, bilateral openings facilitate insertion of a wide array of instruments. An injection cannula provides alternative ventilation. And a special matte finish prevents reflections, making the operating laryngoscope an ideal choice for laser procedures. Laterally placed suction tubes provide additional smoke evacuation without obstructing the view.

Available in two sizes, the WEERDA Distending Operating Laryngoscope adjusts to the most exacting anatomical requirements of different patients.

To learn more, call Karl Storz at **(800) 421-0837** or contact your local Karl Storz representative.

KARL STORZ GmbH & Co. KG
Mittelstraße 8, D-78532 Tuttlingen, Germany
Postfach 230, D-78503 Tuttlingen, Germany
Telefon: (07461) 70 80
Telefax: (07461) 70 81 05

KARL STORZ Endoscopy-America, Inc.
600 Corporate Pointe
Culver City, CA 90230-7600
Telephone: (310) 338-8100
(800) 421-0837
Telefax: (310) 410-5530

KARL STORZ Endoscopy-Canada, Inc.
438 University Avenue, Suite 1800
Toronto, Ontario, Canada M5G 2K8
Telephone: (416) 596-9900
(800) 268-4880 (English)
(800) 361-7388 (Francais)
Telefax: (416) 596-9333

KARL STORZ Endoscopia Latino America
815 N.W. 57th Ave., Ste. No. 480
Miami, FL 33126-2042, USA
Telefono: (305) 262-8980
Telefax: (305) 262-8986

E-mail: karlstorz-marketing@karlstorz.de
Internet: http://www.karlstorz.de
http://www.karlstorz.com

STORZ
KARL STORZ ENDOSCOPY
THE DIAMOND STANDARD

© 2000 Karl Storz Endoscopy-America, Inc.

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

— Founded in 1892 —

Editor

BRIAN F. McCABE, MD
Iowa City, Iowa

Associate Editors

CHARLES D. BLUESTONE, MD
Pittsburgh, Pa

GEORGE A. GATES, MD
Seattle, Wash

OLLIVIER LACCOURREYE, MD
Paris, France

Board of Editors

JOHN G. BATSAKIS, MD	Houston, Tex; Kalkaska, Mich
JOEL M. BERNSTEIN, MD, PhD	Buffalo, NY
ANDREW BLITZER, MD, DDS	New York, NY
DERALD E. BRACKMANN, MD	Los Angeles, Calif
POUL BRETLAU, MD, DMSc	Copenhagen, Denmark
ITZHAK BROOK, MD	Washington, DC
RICHARD A. BUCKINGHAM, MD	Park Ridge, Ill
RICHARD A. CHOLE, MD, PhD	St. Louis, Mo
ALFIO FERLITO, MD	Udine, Italy
UGO FISCH, MD	Zurich, Switzerland
IMRE FRIEDMANN, MD, ScD	Middlesex, England
BRUCE GANTZ, MD	Iowa City, Iowa
LEE A. HARKER, MD	Omaha, Neb
JEFFREY P. HARRIS, MD, PhD	San Diego, Calif
GERALD B. HEALY, MD	Boston, Mass
WILLIAM F. HOUSE, MD	Los Angeles, Calif
STEVEN K. JUHN, MD	Minneapolis, Minn
TIMOTHY T. K. JUNG, MD, PhD	Loma Linda, Calif
DAVID W. KENNEDY, MD	Philadelphia, Pa
FRANK E. LUCENTE, MD	New York, NY
WILLIAM L. MEYERHOFF, MD, PhD	Dallas, Tex
JOSEF MILLER, PhD	Ann Arbor, Mich
AAGE R. MØLLER, PhD	Dallas, Tex
WILLIAM W. MONTGOMERY, MD	Boston, Mass
EUGENE N. MYERS, MD	Pittsburgh, Pa
JOSEPH B. NADOL, Jr, MD	Boston, Mass
YASUYA NOMURA, MD	Tokyo, Japan
LORNE S. PARNES, MD	London, Canada
JAI H. RYU, PhD	Winston-Salem, NC
JACOB SADÉ, MD	Tel Aviv, Israel
ISAMU SANDO, MD	Pittsburgh, Pa
CLARENCE T. SASAKI, MD	New Haven, Conn
ROBERT T. SATALOFF, MD, DMA	Philadelphia, Pa
RICHARD J. H. SMITH, MD	Iowa City, Iowa
HEINZ R. STAMMBERGER, MD	Graz, Austria
DEAN M. TORIUMI, MD	Chicago, Ill
JAN E. VELDMAN, MD, PhD	Utrecht, The Netherlands
BRUCE M. WENIG, MD	Bronx, NY
T. J. YOO, MD, PHD	Memphis, Tenn

Software Review and Computer Applications Editor

KEVIN T. KAVANAGH, MS, MD — Somerset, Ky

Imaging Editor

YUTAKA SATO, MD — Iowa City, Iowa

Book Review Editor

DENNIS G. PAPPAS, MD — Birmingham, Ala

Emeritus Board Members

WESLEY H. BRADLEY, MD	Albany, NY
JACK D. CLEMIS, MD	Chicago, Ill
SEYMOUR COHEN, MD	Los Angeles, Calif
CHARLES W. CUMMINGS, MD	Baltimore, Md
PATRICK J. DOYLE, MD	Vancouver, Canada
ARNDT J. DUVALL III, MD	Minneapolis, Minn
JOHN M. FREDRICKSON, MD	St Louis, Mo
RICHARD R. GACEK, MD	Syracuse, NY
JEROME C. GOLDSTEIN, MD	Lake Worth, Fla
SIR DONALD F. N. HARRISON, MD PhD	London, England
SAM E. KINNEY, MD	Cleveland, Ohio
JOHN A. KIRCHNER, MD	New Haven, Conn
DAVID J. LIM, MD	Los Angeles, Calif
FRED H. LINTHICUM, Jr, MD	Los Angeles, Calif
MICHEL PORTMANN, MD	Bordeaux, France
LORING W. PRATT, MD	Waterville, Me
FRANK N. RITTER, MD	Ann Arbor, Mich
ROBERT J. RUBEN, MD	Bronx, NY
WILLIAM H. SAUNDERS, MD	Columbus, Ohio
RUEDIGER THALMANN, MD	St Louis, Mo
LESTER D. R. THOMPSON, MD	Washington, DC
HARVEY M. TUCKER, MD	Cleveland, Ohio
PAUL H. WARD, MD	Los Angeles, Calif

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

— Founded in 1892 —

ANNALS
PUBLISHING CO.

EDITOR'S OFFICE

Brian F. McCabe, MD
Department of Otolaryngology –
Head & Neck Surgery
University Hospitals & Clinics
Iowa City, IA 52242
(319) 356-2310

BUSINESS OFFICE

Annals Publishing Company
4507 Laclede Avenue
St Louis, MO 63108
(314) 367-4987
Fax (314) 367-4988
E-mail: manager@Annals.com

STAFF

Managing Editor
Michol L. Sheridan

Business Manager
Kenneth A. Cooper, Jr

Chief Copyeditor
Edna Hayes Harrison

Copyeditor
Scott A. Bailey

Circulation Manager
Kevin R. Cooper

Editorial Assistant
Sarah G. Keith

ADVERTISING REPRESENTATIVES

— East & Midwest —
Cunningham Associates
180 Old Tappan Rd
Old Tappan, NJ 07675
(201) 767-4170
Fax (201) 767-8065
JPattis@cunnasso.com

— West —
Charles B. Healy
PO Box 5213
Palm Springs, CA 92263
(760) 320-0118

EDITORIAL CONTENT

The *ANNALS* publishes original manuscripts of clinical and research importance in otolaryngology – head and neck medicine and surgery, bronchoesophagology, laryngology, head and neck oncology and surgery, plastic and reconstructive surgery, pediatric otolaryngology, audiology, speech pathology, and related specialties. In-depth studies (supplements), papers of historical interest, reviews of computer software and applications in otolaryngology are also published, as well as imaging, pathology, and clinicopathology studies, book reviews, and letters to the editor. All papers and supplements are peer-reviewed. The *ANNALS* is the official journal of the American Laryngological Association and the American Broncho-Esophagological Association.

SUBSCRIPTIONS (INCLUDES ON-LINE SUBSCRIPTION)

2001 Annual Subscription Rates	*USA*	*International Surface Mail (All Regions)*
Institution*	$240.00	$265.00
Individual†	170.00	195.00
Resident/Student†	112.00	129.00

*Institutional (multiple-reader) subscription rates apply to public and private libraries, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and commercial and private institutions and organizations.

†Individual, resident, and student subscriptions must be in the individual's name and must be billed to and paid for by the individual.

Airmail rates are available upon request.

Visa, Mastercard, and American Express are accepted as payment.

WEB SITE

http://www.Annals.com

AVAILABILITY OF CD-ROM

CD-ROMs are available for each volume in March of the following year. The 1998 volume was the first on CD-ROM. Current subscribers can purchase a CD-ROM at $76.00 for an individual and $99.00 for an institution. Orders placed by non-subscribers are $175.00 for an individual and $245.00 for an institution.

CLAIMS & BACK ISSUES

Claims. Claims for missing issues must be received within 60 days of the month of publication for the United States and Canada, and 180 days for other countries. Single copy prices will be charged on missing issues claimed after that time.

Back Issues. A comprehensive inventory of back issues and supplements is available. Prices are furnished on request.

Change of Address. Address changes must be received 45 days before the effective date so that the subscription will be received without interruption.

MICROFILM

The *ANNALS* is available in microform from University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106.

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY (ISSN 0003-4894) is published monthly by Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108. Periodicals postage paid at St Louis, Mo. POSTMASTER: Send address changes to *ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY*, c/o Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108-2103.

COPYRIGHT© 2001, ANNALS PUBLISHING COMPANY

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

VOLUME 110 FEBRUARY 2001 NUMBER 2

CONTENTS

OTOLOGY

Twenty-Year Report of the First Auditory Brain Stem Nucleus Implant
William F. House, DDS, MD; William E. Hitselberger, MD 103

Hearing Improvement After Therapy for Hyperlipidemia in Patients With Chronic-Phase Sudden Deafness
Yoshimasa Kojima, MD; Shigehiko Ito, MD; Nobuhiko Furuya, MD 105

Effects of Exposing the Opened Endolymphatic Sac to Large Doses of Steroids to Treat Intractable Meniere's Disease
Tadashi Kitahara, MD, PhD; Noriaki Takeda, MD, PhD; Yasuo Mishiro, MD; Takanori Saika, MD, PhD; Munehisa Fukushima, MD; Shin-ichi Okumura, MD, PhD; Takeshi Kubo, MD, PhD 109

Inner Ear Fluid Volumes and the Resolving Power of Magnetic Resonance Imaging: Can It Differentiate Endolymphatic Structures?
Richard A. Buckingham, MD; Galdino E. Valvassori, MD 113

Poly(Adenosine Diphosphate–Ribose) Synthetase Inhibitor 3-Aminobenzamide Alleviates Cochlear Dysfunction Induced by Transient Ischemia
Keiji Tabuchi, MD; Zenya Ito, MD; Shigeki Tsuji, MD; Akiko Nakagawa, MD; Fujiko Serizawa, MD; Akira Hara, MD; Jun Kusakari, MD 118

Changes in Permeability of Strial Vessels Following Vibration Given to Auditory Ossicle by Drill
Morihiro Seki, MD; Hiroe Miyasaka, MD; Hideo Edamatsu, MD; Kensuke Watanabe, MD 122

Vestibular Findings in Professional Divers
Zohara Sharoni, MA; Avi Shupak, MD; Orna Spitzer, MA; Zohar Nachum, MD; Natan Gadoth, MD 127

Immortalization of Rat Middle Ear Epithelial Cells by Adeno 12–SV40 Hybrid Virus
Shigehiro Ueyama, MD; Sunji Jin, MD; Johng S. Rhim, MD; Tomoyo Ueyama, MD; David J. Lim, MD 132

NOSE AND PARANASAL SINUSES

Long-Term Study of Endonasal Duraplasty and Review of the Literature
Bernhard Schick, MD; Rainer Ibing; Dominik Brors, MD; Wolfgang Draf, MD, FRCS 142

Correlation Between Microbiology and Previous Sinus Surgery in Patients With Chronic Maxillary Sinusitis
Itzhak Brook, MD, MSc; Edith H. Frazier, MSc 148

LARYNX

Loss of Secondary Esophageal Peristalsis Is Not a Contributory Pathogenetic Factor in Posterior Laryngitis
Seckin O. Ulualp, MD; Chengming Gu, MD; Robert J. Toohill, MD; Reza Shaker, MD 152

Thyroarytenoid Muscle: Functional Subunits Based on Morphology and Muscle Fiber Typing in Cats
Rui Imamura, MD; Yoshikazu Yoshida, MD; Hiroyuki Fukunaga, MD;
Tadashi Nakashima, MD; Minoru Hirano, MD 158

PHARYNX AND ESOPHAGUS

Retropharyngeal Autologous Fat Transplantation for Congenital Short Palate: A Nasometric Assessment of Functional Results
Philippe H. Dejonckere, MD, PhD; Hans A. van Wijngaarden, MD, PhD 168

Comparison of the Microanatomical Distributions of Macrophages and Dendritic Cells in Normal and Diseased Tonsils
Janet L. Gorfien, MA; Bernice Noble, PhD; Linda Brodsky, MD 173

Quantitative Assessment of the Pharyngeal Airway by Dynamic Magnetic Resonance Imaging in Obstructive Sleep Apnea Syndrome
Katsuhisa Ikeda, MD; Masaki Ogura, MD; Takeshi Oshima, MD; Hideaki Suzuki, MD;
Shuichi Higano, MD; Shoki Takahashi, MD; Hajime Kurosawa, MD; Wataru Hida, MD;
Hiroo Matsuoka, MD; Tomonori Takasaka, MD 183

Effect of Cigarette Smoking on Gastropharyngeal and Gastroesophageal Reflux
Conrad F. Smit, MD; Marcel P. Copper, MD, PhD; Justin A. M. J. van Leeuwen, MD;
Ivo G. Schoots, MD; Laki D. Stanojcic, MD 190

Bifid Epiglottis Associated With Joubert's Syndrome
Myung-Whun Sung, MD; Jeong-Whun Kim, MD; Kwang Hyun Kim, MD 194

Epiglottic Tuberculosis: Differential Diagnosis and Treatment. Case Report and Review of the Literature
Bernhard Richter, MD; Milo Fradis, MD; Gabriele Köhler, MD; Gerd Jürgen Ridder, MD 197

IMAGING CASE STUDY OF THE MONTH

Chondrosarcoma of the Nasal Septum
Pasquale Blotta, MD; Francesco Carinci, MD; Stefano Pelucchi, MD;
Luigi Cavazzini, MD; Alessandro Martini, MD 202

MISCELLANEOUS

Information for Subscribers 2a
Instructions to Authors 6a
Index to Advertisers 10a

FELLOWSHIP IN LARYNGOLOGY, PHONOSURGERY AND VOICE DISORDERS

HARVARD
MEDICAL SCHOOL

Available July 1, 2003. The Department of Otology and Laryngology is seeking applicants for a one-year clinical fellowship in laryngology, phonosurgery, and management of the professional voice. The curriculum will provide a wide range of experiences, including phonomicrosurgery (cold instruments and lasers), laryngeal framework surgery, laryngeal electromyography, and the use of botulinum toxin injections for spasmodic dysphonia. The fellow will participate in the management of voice disorders as a member of a multidisciplinary team that includes full exposure to state-of-the-art voice laboratory facilities. The curriculum is designed so that upon completion, the fellow will have sufficient background to establish an independent voice disorders program at his or her home institution. There are numerous clinical and basic science research opportunities available as well as the option to work collaboratively with local music conservatories. Certification by the American Board of Otolaryngology or eligibility is a prerequisite.

Direct inquiries to: Steven M. Zeitels, MD, Director, Division of Laryngology, Massachusetts Eye and Ear Infirmary,
243 Charles Street, Boston, MA 02114;
Phone: (617) 573-3557, Fax: (617) 573-3068

A136

KAISER PERMANENTE
More people turn to us for good health.

DENVER, COLORADO OTOLARYNGOLOGY

The Colorado Permanente Medical Group is seeking a Board-eligible/certified otolaryngologist for a stimulating referral practice in a prepaid group setting. Salaried position with excellent benefits. Denver offers abundant four-season outdoor activities enhanced by many community amenities.

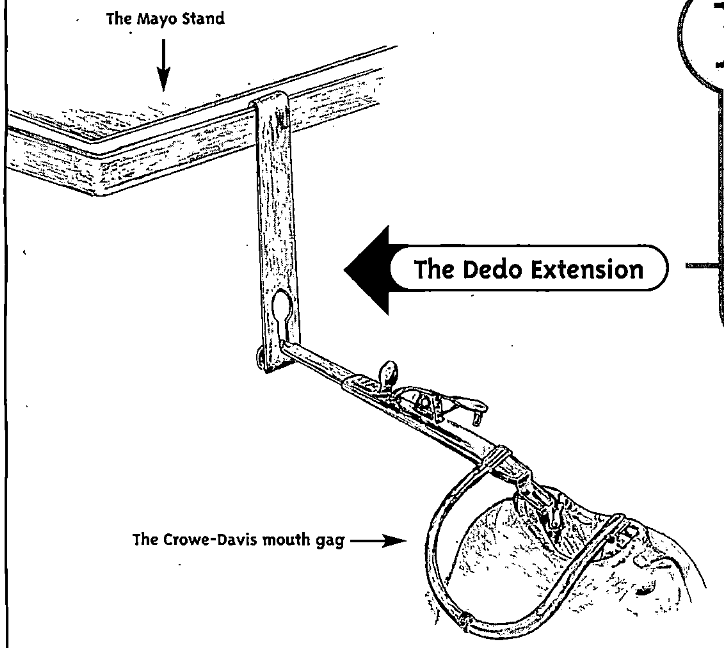
Contact:

Physician Recruitment
Colorado Permanente Medical Group
10350 E Dakota Ave
Denver, CO 80231
Phone: 303-344-7302
Fax: 303-344-7818

EOE,M/F,V/H

A187

Reach More Patients.



People come in different shapes and sizes. The same piece of equipment does not fit them all. Make oropharyngeal surgery easier with a simple extension. The CANT Corporation has created the Dedo Extension that fits between the Mayo Stand and the Crowe-Davis mouth gag so that it can be adjusted to fit larger patients. The Dedo Extension - a simple but effective solution.

Applicable Procedures
- T&A's
- Uvuloplasty
- Palatoplasty
- All oropharyngeal procedures using a Crowe-Davis mouth gag

cantcorpcan@begneaud.com 337•231•5830

CANT
CORPORATION

INSTRUCTIONS TO AUTHORS

SUBMISSION

Send manuscripts to Editor Brian F. McCabe, MD, Department of Otolaryngology–Head and Neck Surgery, The University of Iowa Hospitals and Clinics, Iowa City, IA 52242. Original manuscripts dealing with clinical and scientific aspects of otolaryngology, bronchoesophagology, head and neck medicine and surgery, maxillofacial and plastic surgery, audiology, speech pathology, and related specialties are considered for publication. All materials submitted for publication undergo peer review. All manuscripts must be accompanied by a signed copyright transfer (see "Copyright," below). Include a self-addressed, stamped envelope for notification of receipt of manuscript.

Written permission from both senior author and publisher must be provided to the *Annals* in order to republish tables or illustrations copyrighted elsewhere. Submit this permission with the manuscript.

Papers are scheduled for publication in chronological order of acceptance. Manuscripts received in improper form require longer production time. Manuscripts are edited in accordance with the *AMA Manual of Style*, 9th edition (1998), and with the *Uniform Requirements for Manuscripts Submitted to Biomedical Journals* (*Ann Intern Med* 1988;108:258-65).

MANUSCRIPT PREPARATION

Limit papers to a size that will make up to no more than six printed pages, figuring three double-spaced typewritten pages of text to one typeset page; see the journal for estimating space required for references, illustrations, and tables. If a manuscript of greater length is accepted for publication by the Editor, all typeset pages over six are charged to the author at the publisher's cost of $150 per page. Submit an original and two copies of the manuscript, typed on white bond paper with margins of at least 25 mm (1 in), double-spaced throughout, including abstract, references, tables, and legends. Use a type size no smaller than 10 points, preferably 12. Begin each component on a new page in the following sequence: title page, abstract, text, acknowledgments, references, tables, and figure legends. Number pages consecutively in the upper right corner, beginning with the title page. Avoid the use of staples.

The author is responsible for all statements in the paper, as approved on the copyedited galley proofs. Alterations made by the author after the paper has been typeset are charged to the author.

Use standard abbreviations given in *Uniform Requirements*. Express all measurements in metric terms; if original measurements were made in another system, include these parenthetically. Plot audiograms according to ISO standards. Use generic names whenever possible.

Title page must include 1) a concise but informative title, worded to facilitate indexing; 2) an abbreviated form of the title to be used as a running head; 3) authors' full names (no more than seven authors are permitted) and no more than two academic degrees per author; 4) department(s) and institution(s) to which the work is attributed, with authors' present affiliations and addresses, if different, separately noted; 5) statement of grant or other support; 6) name and address of author to whom reprint requests should be sent; and 7) name, address, telephone and fax numbers, and, if possible, e-mail address of corresponding author. Manuscripts that report animal research performed in the United States must carry the following statement on the title page: "This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of __________ University (or institution)."

Abstracts contain no more than 150 words and state the goal of the study, primary procedures, main findings, and principal conclusions.

Key Words, chosen as far as possible from the National Library of Medicine *Medical Subject Headings*, are listed after the abstract.

References, double-spaced, are numbered consecutively in the order in which they are cited in the text. Primary references should be used whenever possible. The author is charged $1.50 for each reference over 30. Use the style of references given in *Uniform Requirements* or a current issue of the *Annals*. Include the names of all authors and the inclusive page numbers of an article. If a manuscript accepted but not yet published is included in the reference list, give the accepting journal's name, followed by "in press." Manuscripts still in review or not yet accepted formally are cited within the text as "unpublished observations." A reference to a personal communication is also placed in the text, accompanied by a date (year). Papers presented at scholarly meetings but not published are considered "unpublished observations." Papers published only in abstract form are listed as references with "[Abstract]" after the title.

Tables should be on separate sheets, numbered consecutively and headed by a concise title. Put explanatory matter in footnotes. Tables are adjuncts to the text and should not repeat material presented therein. The cost of typesetting tables is billed to the author.

Illustrations must be submitted in three complete sets, unmounted. Only professional-quality glossy photographs and black-and-white line drawings are acceptable. Computer-generated graphs are acceptable only if they are clear and will reproduce well. Letters, numbers, and symbols should be clear and of uniform size throughout, large enough that each item is legible when reduced for publication. Put legends (detailed explanations) to the photographs on a separate page in the manuscript. Affix a label to the reverse side of each illustration indicating figure number, first author's name, and top of the figure. For radiographs, photomicrographs, CT scans, etc, give indications for maximum cropping and labeling on an overlay or photocopy. Cite each figure in the text in consecutive order. Written permission from identifiable subjects is required. The cost of preparing illustrations for publication (sizing to fit journal, and preparation of negatives for printing) is charged to the author without exception. Color illustrations are accepted; cost estimates for color separations and printing are provided on an individual basis. Illustrations should enhance, not repeat, material presented in the manuscript, and should be kept to a minimum.

REVIEWS OF SOFTWARE AND COMPUTER APPLICATIONS

Programs submitted for review must be compatible with an IBM or Macintosh computer, and the author must specify hardware and system requirements. The following operating systems will be supported: MS-DOS; MS-DOS with Windows; and MAC-OS. Submit the software on either a 3.5" or 5.25" disk. If the program is a template, submit also a runtime version of the source program. Software considered for review includes educational software, artificial intelligence software to aid in the diagnosis of otolaryngic disorders, software for logging operations, software to aid in research, and software that addresses specific problems in otolaryngology. Also, manuscripts reviewing software and computer applications relating directly to otolaryngology are considered for publication. See *Ann Otol Rhinol Laryngol* 1991; 100:876 for specific instructions.

SUPPLEMENTS

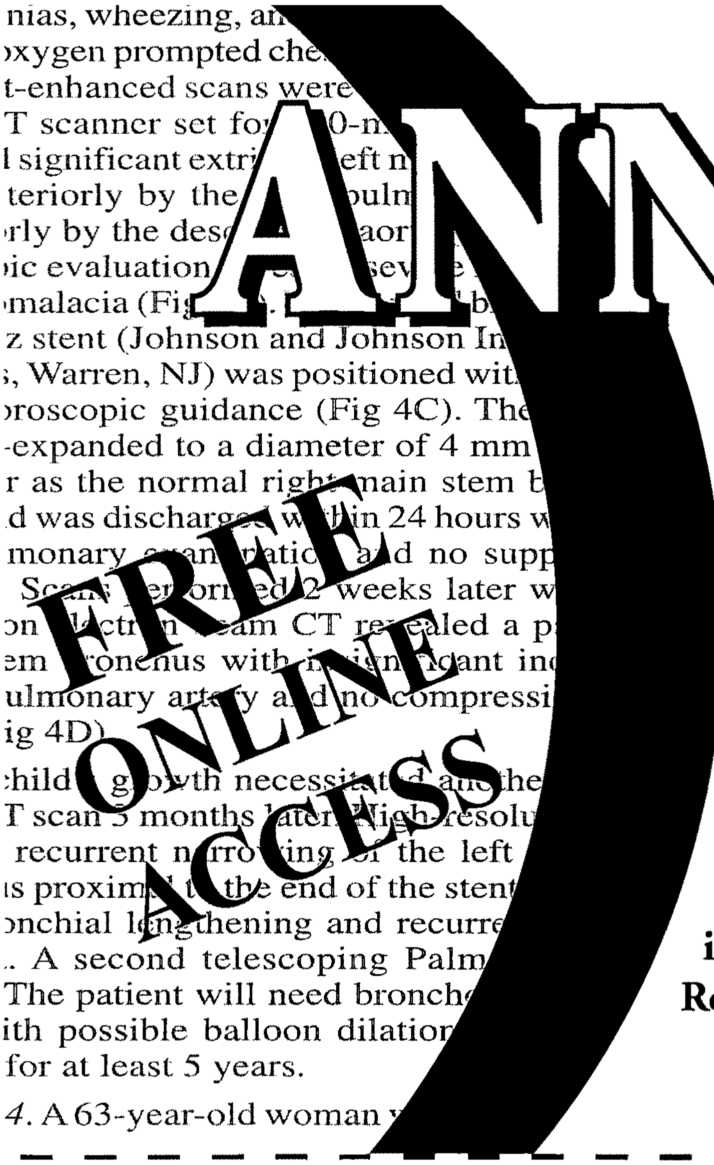
A manuscript too long for inclusion in the *Annals* (over 12 typeset pages) may be published as a supplement if approved by the Editor. All costs are borne by the author and estimates are provided upon request. Supplements have the advantages of separate identification and rapid publication, but undergo the same critical review as journal articles.

REPRINTS

Rates are quoted when galley proofs are sent to the author. Orders are signed by the author and returned with the proofs.

COPYRIGHT

The following statements should accompany the manuscript, signed by all authors (only original signatures are acceptable): 1) "This paper has not been published or submitted for publication elsewhere." 2) [*For papers with more than one author*] "I have contributed substantively to the development of the content of this paper and have agreed to have my name listed as an author in the final, revised version." 3) "In consideration of the *Annals of Otology, Rhinology & Laryngology* taking action in reviewing and editing my (our) submission, I hereby transfer, assign, or otherwise convey all copyright ownership to Annals Publishing Company in the event such work is published in the *Annals of Otology, Rhinology & Laryngology*."


FREE
ONLINE
ACCESS
nias, wheezing, an
oxygen prompted che
t-enhanced scans were
T scanner set fo
l significant extr
teriorly by the
rly by the des
ic evaluation
malacia (Fi
z stent (Johnson and Johnson In
, Warren, NJ) was positioned wit
roscopic guidance (Fig 4C). The
-expanded to a diameter of 4 mm
r as the normal right main stem b
d was discharged within 24 hours w
monary
weeks later w
on
CT revealed a p
em
ulmonary
ig 4D).
hild
growth necessitated another
T scan 5 months later. High-resolu
recurrent narrowing of the left
is proxim
the end of the sten
onchial lengthening and recurre
. A second telescoping Palm
The patient will need bronch
ith possible balloon dilatio
for at least 5 years.
4. A 63-year-old woman

ANNALS

OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

Bringing you the most relevant developments and breakthroughs in otolaryngology—in time for you to put them into practice.

For the first time the *ANNALS* online is FREE to subscribers all over the world. Read it *before* you receive your journal!

http://www.annals.com

YES! Enter my subscription for 12 monthly issues of *ANNALS* for the calendar year January-December 2001. *(Please print.)*

☐ New ☐ Renewal

Name ______________________ E-mail ______________________

Address ______________________

City ______________ State ______________ ZIP ______________

Country ______________________ Specialty ______________________

2001 Annual Subscription Rates (includes online subscription)	USA	International Surface Mail (All Regions)
Institution	$240.00	$265.00
Individual	$170.00	$195.00
Resident/Student	$112.00	$129.00

☐ Payment enclosed* ☐ Visa ☐ Mastercard ☐ American Express

Card No. ______________________ Expiration Date ______________

Signature ______________________ Print name as it appears on card ______________________

*Checks must be in US dollars and drawn on a US bank payable to Annals Publishing Company.

Fax to (314) 367-4988 or mail to ANNALS PUBLISHING COMPANY, 4507 LACLEDE AVENUE, ST LOUIS, MO 63108, USA
E-mail: Manager@Annals.com • http://www.annals.com

Ann Otol Rhinol Laryngol 110:2001

TWENTY-YEAR REPORT OF THE FIRST AUDITORY BRAIN STEM NUCLEUS IMPLANT

WILLIAM F. HOUSE, DDS, MD
NEWPORT BEACH, CALIFORNIA

WILLIAM E. HITSELBERGER, MD
LOS ANGELES, CALIFORNIA

We present the 20-year case report of an auditory implant placed on the dorsal cochlear nucleus with long-term electrical stimulation in a patient with neurofibromatosis 2. The patient has continued to use her implant daily for 20 years, and it has greatly enhanced her quality of life. There have been no adverse sequelae.

KEY WORDS — auditory brain stem nucleus implant, hearing rehabilitation.

INTRODUCTION

Neurofibromatosis type 2 (NF2) often requires removal of bilateral neuromas, resulting in total deafness. It can be a dominant hereditary problem or occur as an isolated genetic abnormality. During the 1960s, we had occasion to see several families with this unfortunate malady. Our concept of treatment at first was to repeatedly partially debulk the tumors in such a way as to avoid the seventh and eight nerves and thus avoid increased intracranial pressure and hearing loss as the tumors enlarged. We were soon disappointed to find that even this partial removal of the tumor resulted in loss of hearing.

Linthicum[1] studied the specimens that were removed and found that the NF2 tumors invaded the eighth nerve and spread the nerve fibers throughout the tumor. This was the reason that debulking the tumor resulted in loss of hearing. This is in contrast to unilateral neuromas that push the auditory part of the eighth nerve aside over the surface of the tumor. This pressure and stretching of the nerve results in gradual loss of hearing. On the other hand, NF2 patients usually retain good hearing until the tumors are large enough to stretch the nerve fibers as they course through the tumor.

In the early 1960s, our experience with cochlear implants and acoustic neuroma surgery prompted us to consider placing an electrode on the cochlear nucleus, since it was readily accessible on the surface of the brain stem at the time of the tumor removal.

Our principal concern regarding this procedure was the possibility of vital sign changes that could result from electrical stimulation of the surface of the brain stem. We reasoned, however, that we had not seen vital sign changes with bipolar cauterization of small vessels on the surface of the brain stem. Also, we had patients who were using monopolar cochlear implants. The current flow was from the perilymph of the cochlea to the fluid around the spiral ganglion cells and out through the internal auditory canal and finally, probably, along the blood vessels (path of least electrical resistance) to the return electrode in the temporalis muscle. We had never observed vital sign changes in these patients.

CASE REPORT

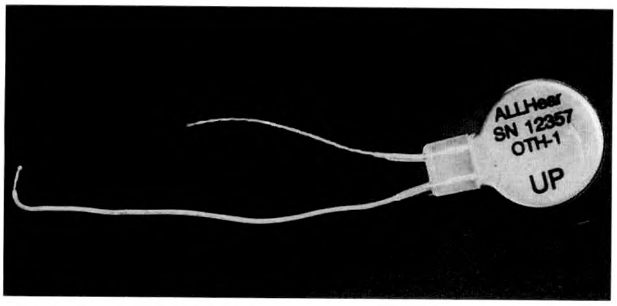
In 1964, a right acoustic neuroma was diagnosed and removed by a translabryinthine approach. After this surgery, the hearing in the left ear was normal, and the right ear had no hearing as a result of the tumor removal. In 1976, because of a progressive hearing loss in the left ear, an intracanicular tumor was discovered. A middle fossa decompression of the internal auditory canal was done in a attempt to slow the progression of the hearing loss. By 1979, the tumor had grown to 3 cm, and the hearing loss had dropped to 85 dB with 48% discrimination. There was some early hydrocephalus, and it was deemed imperative that the tumor be removed.

The patient was counseled about the tumor removal, and the possibility of a cochlear nucleus electrode was explained. She was told that this had not been done before and that the risks were unknown, since long-term electrodes had never been placed on the brain stem.

On May 24, 1979, the left acoustic neuroma was totally removed, and the facial nerve was dissected away from the tumor. After the tumor removal, the brain stem area was explored, and the remnant of the eighth nerve was identified in the area of the dorsal cochlear nucleus. A platinum electrode pair with 0.5-mm balls separated by 1.5 mm was then placed on the cochlear nucleus and pushed through the ependyma in the region of the opening of the fourth ven-

---

CORRESPONDENCE — William F. House, DDS, MD, 361 Hospital Rd, Suite 327, Newport Beach, CA 92663.



Electrode used in brain stem implant patient. Initially, electrode was hardwired (transcutaneous). It was later converted to coil and magnet type shown here.

tricle, the foramen of Luschka.

The wires were then led out through the mastoid, sutured to the cortical bone, and led out through the top of the incision. The mastoid cavity was filled with abdominal fat, and the incision was closed with subcuticular gut sutures and Mersilene skin sutures. There were no vital sign changes during the procedure, and the patient was allowed to react on the operating room table.

The following day, the patient was awake, alert, and oriented and could read instructions and reply verbally. The patient was returned to the operating room, where full anesthesia personnel and vital sign monitoring were available. Through the hardwired connectors, a 16-kHz carrier was amplitude-modulated with various speech frequencies. The intensity was gradually increased until the patient experienced a sensation of sound.

Fortunately, there were no vital sign changes from this stimulation. There was no facial nerve twitching in the surgically weakened facial nerve. There was no sensation of vertigo. The facial nerve and the brain stem vestibular nuclei are in close proximity to the stimulating electrodes on the dorsal cochlear nucleus, and before the operation we had been concerned that both of these structures would be stimulated. Apparently, the auditory brain stem nuclei are tuned to auditory electrical frequencies and will only respond to these frequencies. The surrounding structures are not tuned to these frequencies and thus do not respond.

During the next week, the patient was stimulated each day with a doctor present and a ventilator available. On the 3-word set comparison (monosyllable versus trochee versus spondee), she attained approximately 75%. Her errors were mainly confusing the 2-syllable words, the trochee and the spondee. The patient continued to experience sounds and found the implant a great help in lipreading. In a week, she was sent home with a 3M/House processor attached to her hardwired electrodes.

The patient continued to use the implant daily. She reported being able to hear her dog bark, a knock on the door, an airplane overhead, and the sound of the sink garbage disposal. She had considerable relief of tinnitus. She noted increasing discrimination, and the unit was of considerable help in lipreading. After a time, she developed a rather strange sensation in the front of her left thigh when the unit was activated. We were not sure of the reason for this, since it did not seem to follow a neurologic pattern. She was requested to stop wearing the unit to see if this would clear up.

In June of 1985, under local anesthesia, the transcutaneous plug was removed, and a titanium-enclosed internal coil (see Figure) was connected to the electrode wires and embedded under the postauricular scalp. This made it much easier for the patient to wear the 3M/House processor. The problem with the sensation in the left thigh disappeared.

The patient has subsequently had several other surgeries for tumor removal, including a left facial nerve neuroma.

AUDIOLOGICAL TESTS

The patient continues to use her implant daily for 14 to 16 hours, and her hearing has now stabilized. The most recent tests were in June 1990 and the results were as follows. The warble-tone thresholds were 35 dB at 250 Hz, 50 dB at 500 Hz, 60 dB at 1,000 Hz, 60 dB at 2,000 Hz, 75 dB at 3,000 Hz, and 80 dB at 4,000 Hz. The spondee test outcome was 35 dB at speech detection threshold; the upper level limit was not tested. On the monosyllable-trochee-spondee test, her scores were 46% on words and 96% on stress. Her environmental sound score was 45%. She had 55% of the key words correct on the CID Everyday Sentences Test.

SUMMARY

This patient is the first to have long-term stimulation (20 years) of the brain stem dorsal auditory nucleus. There has been no deterioration of the stimulation since it stabilized at about 1 year.

Many auditory brain stem implants throughout the world are now in successful use. This case indicates that these implants will be a permanent rehabilitation for these patients.

REFERENCE

1. Linthicum FH Jr. Unusual audiometric and histologic findings in bilateral acoustic neurinomas. Ann Otol Rhinol Laryngol 1972;81:433-7.

Ann Otol Rhinol Laryngol 110:2001

HEARING IMPROVEMENT AFTER THERAPY FOR HYPERLIPIDEMIA IN PATIENTS WITH CHRONIC-PHASE SUDDEN DEAFNESS

YOSHIMASA KOJIMA, MD

SHIGEHIKO ITO, MD

NOBUHIKO FURUYA, MD

ICHIHARA CITY, JAPAN

Hearing in patients with chronic-phase sudden deafness and associated hyperlipidemia tends to improve with therapy for hyperlipidemia. We studied 12 patients with unilateral sudden deafness and hyperlipidemia in whom more than 1 month had elapsed since the onset of the hearing disturbance. The disturbance was considered to be irreversible without therapy. The 4 men and 8 women ranged in age from 32 to 73 years, with a mean age of 54.3 years. The hearing disturbance was evaluated by measuring the air conduction thresholds at 125 to 8,000 Hz with pure tone audiometry. Hyperlipidemia was diagnosed when the total blood cholesterol level was 230 mg/dL or greater. The therapy for hyperlipidemia consisted of diet therapy and the administration of antilipemic agents. The hearing level was measured both before therapy and when the total blood cholesterol level had decreased to less than 230 mg/dL. After therapy, the mean hearing level had improved significantly (Wilcoxon's test, $p < .05$) at each of 125, 250, 500, and 2,000 Hz, but the changes in the level were not significant at 1,000, 4,000, or 8,000 Hz. Our results suggest that with therapy for hyperlipidemia, hearing tends to improve in patients with chronic-phase sudden deafness and associated hyperlipidemia, even when more than 1 month has elapsed since the onset of the presumably otherwise-irreversible hearing disturbance.

KEY WORDS — hyperlipidemia, sudden deafness.

INTRODUCTION

Sudden hearing loss can be attributed to various pathological conditions and lesions. A circulatory disturbance is one possible cause of sudden deafness.[1,2] Some patients with hearing loss improve when treated soon after onset. However, no hearing improvement can be expected in patients in the chronic phase, in whom more than 1 month has elapsed after the onset, and the hearing disturbance in these patients is presumed to be irreversible. In the present study, we treated patients with chronic-phase sudden deafness and associated hyperlipidemia, which latter suggested the presence of a circulatory disturbance. We noted a hearing improvement after the therapy for hyperlipidemia, even though more than 1 month had passed since the onset of sudden deafness.

SUBJECTS

The subjects were 12 patients who had both unilateral sudden deafness and hyperlipidemia. They visited our department complaining of hearing loss and tinnitus between March 1995 and October 1996. They were 4 men and 8 women, ranging in age from 32 to 73 years, with a mean age of 54.3 years. From the patients' histories of hearing loss, we surmised that the onset of sudden deafness had occurred more than 1 month before the first visit. Audiometry was performed for both the air and bone conduction hearing thresholds. The air conduction hearing thresholds were measured at 125 to 8,000 Hz, and the bone conduction hearing thresholds were measured at 250 to 4,000 Hz. In all subjects, the air-bone gap was 5 dB or less at 250 to 4,000 Hz. The diagnosis of sensorineural hearing loss was established when at least 1 threshold was 30 dB or greater among the air conduction hearing thresholds at 250 to 4,000 Hz, which were measured with pure tone audiometry. The hearing loss in all subjects was unilateral, with the hearing thresholds on the uninvolved side being 20 dB or less at 250 to 4,000 Hz.

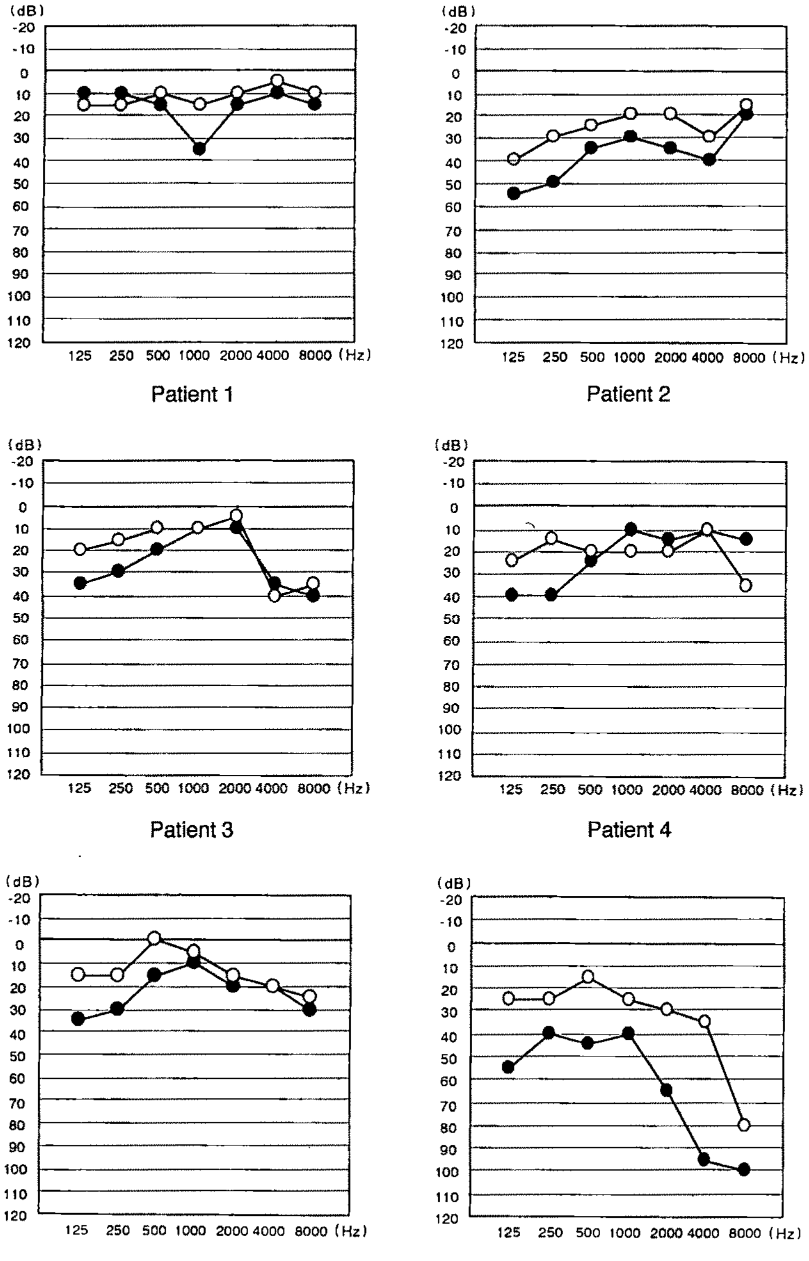
The presence of associated hyperlipidemia (total blood cholesterol level of 230 mg/dL or greater) that

TOTAL BLOOD CHOLESTEROL LEVELS

Patient	*Age (y)*	*Sex*	*Before Treatment (mg/dL)*	*After Treatment (mg/dL)*
1	32	F	231	194
2	62	F	270	205
3	52	F	251	174
4	54	F	258	187
5	42	F	258	217
6	62	M	238	218
7	73	F	275	199
8	53	F	252	214
9	66	M	231	207
10	49	M	238	182
11	55	M	233	212
12	47	F	231	225

From the Department of Otolaryngology, Teikyo University School of Medicine Ichihara Hospital, Ichihara City, Japan.

CORRESPONDENCE — Yoshimasa Kojima, MD, Dept of Otolaryngology, Teikyo University School of Medicine, Ichihara Hospital, 3426-3 Anesaki, Ichihara City, Chiba Prefecture, 299-0111, Japan.



Patient 1

Patient 2

Patient 3

Patient 4

Patient 5

Patient 6

Fig 1. Audiograms made before and after therapy in patients 1 through 6, who showed remarkable improvement in hearing. Closed circles indicate hearing levels before therapy, and open circles indicate those after therapy. Values are for air conduction hearing.

improved after therapy was confirmed in all patients. Conventional therapy for sudden deafness, such as the administration of steroids and circulatory agents, was not indicated, because more than 1 month had elapsed since the onset of hearing loss. The hearing loss was presumed to be irreversible in all patients.

METHODS

Diet therapy was prescribed for the 6 patients whose total blood cholesterol level was between 230 and 249 mg/dL. Diet therapy and the administration of antilipemic agents (pravastatin sodium) were combined to treat the 6 patients whose total blood cholesterol level was 250 mg/dL or greater. The duration of therapy ranged from 90 to 519 days. When the total blood cholesterol level decreased to less than 230 mg/dL, the therapy for hyperlipidemia was considered successful. Audiometry was then performed to evaluate the posttherapy hearing level. The statistical comparison of the mean pretherapy and posttherapy hearing levels was conducted with Wilcoxon's test, and a p value of less than .05 was considered statistically significant.

RESULTS

The Table shows the total blood cholesterol levels measured before and after therapy in each of the 12 patients. The mean value was 247.2 mg/dL before therapy, and it decreased to 202.8 mg/dL after therapy.

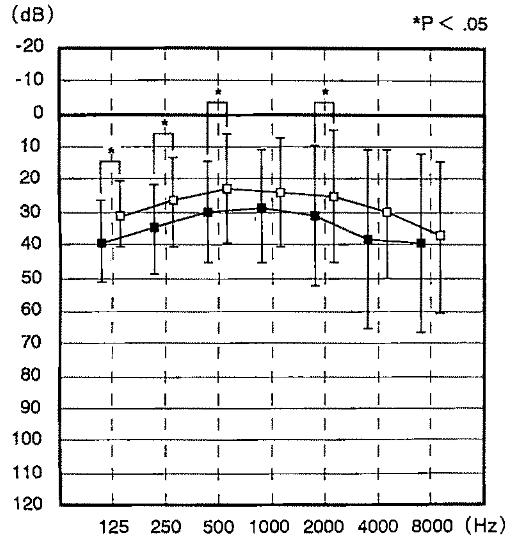


Fig 2. Mean hearing levels of 12 patients before and after therapy. Closed squares indicate mean hearing level before therapy, and open squares indicate those after therapy. Compared with mean hearing level before therapy, mean hearing level improved significantly after therapy at 125, 250, 500, and 2,000 Hz (Wilcoxon test, p < .05).

Hearing improvement by more than 10 dB at more than 1 frequency was observed in 9 of the 12 patients. Figure 1 shows audiograms made before and after therapy in 6 patients who showed marked improvement in hearing. The hearing level at 1,000 Hz improved from 35 to 15 dB with therapy in patient 1. The hearing levels at 125 to 4,000 Hz showed a range of improvement from 10 to 20 dB in patient 2. In patients 3, 4, and 5, the hearing improved at lower frequencies, ie, 125 to 500 Hz. Hearing improvement ranging from 15 to 60 dB was noted in patient 6 at all measured frequencies from 125 to 8,000 Hz.

Figure 2 shows changes that occurred with therapy in the mean hearing level for all 12 patients. Compared with the level measured before therapy, the mean hearing level after therapy was improved significantly at 125, 250, 500, and 2,000 Hz (Wilcoxon's test, $p < .05$). At the other frequencies, the changes in hearing were not significant.

DISCUSSION

Since De Kleyn[3] first described sudden deafness in diabetic patients, there have been several reports that hearing is affected by systemic diseases that cause circulatory disturbances. Rosen et al[4] conducted an epidemiological survey and first demonstrated that hyperlipidemia could be a cause of hearing disturbance. They suggested that the constitution of the diet was an important factor for prevention of coronary disease and hearing disturbance. Spencer[5] also emphasized the potential association of hyperlipidemia with inner ear disorders on the basis of the results of an epidemiological study. Saito[6] and Yamane et al[7] induced a hyperlipidemic state in guinea pigs to investigate the pathophysiology of the inner ear. Although the characteristics and prognosis of hearing loss were described in these studies on patients with diabetic mellitus or hyperlipidemia, hearing improvement has not been reported after aggressive therapy for diabetes mellitus or hyperlipidemia.

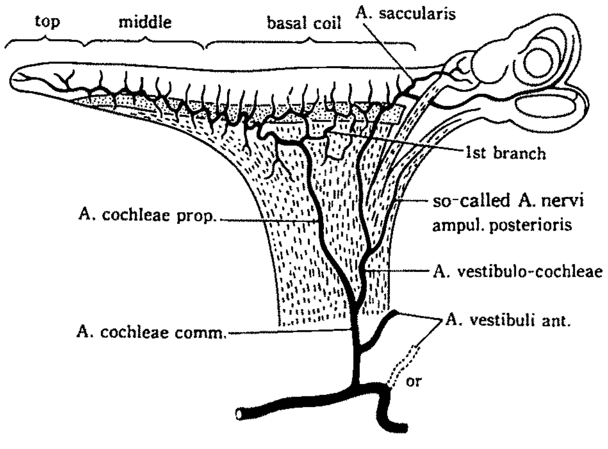


Fig 3. Inner ear arterial system. (Reprinted from Nabeya.[8]) Apical turn of cochlea (top left in Figure) is located in peripheral end of inner ear arterial system.

We encountered a 31-year-old woman (patient 1 in this study) with impaired hearing in March 1995. Although steroids were given under the diagnosis of sudden deafness, her hearing loss did not respond to the therapy and became stable. Diet therapy was started when blood tests revealed associated hyperlipidemia 4 months after the onset of hearing disturbance. Although the objective of the diet therapy was the prevention of a further decrease in hearing and not hearing improvement, her hearing improved with the therapy for hyperlipidemia to an almost normal level, as shown in Fig 1. Expecting similar therapeutic effects on hearing loss, we began to treat associated hyperlipidemia aggressively in patients with sudden deafness.

The reason for this study on 12 patients is described above. Although the mechanism of hearing improvement remains to be investigated, we propose the following hypothesis based on blood circulation in the inner ear. In the 12 patients, the hearing improvement found after therapy was remarkable at the lower frequencies. This finding might be explained by the arterial distribution in the inner ear. Figure 3 shows a schematic drawing of the arterial distribution in the inner ear as described by Nabeya.[8] He classified the arterial distribution in the inner ear into 3 types, including the one shown in Fig 3. In all 3 types, the apical turn of the cochlea is located at the peripheral end of the inner ear arterial system and therefore is considered to be the most susceptible to circulatory disturbances. The results of therapy in our study showed that the hearing loss was reversible. Gussen[2] speculated that in most cases, reversible hearing loss is caused by changes in the stria vascularis. In our patients in the pretherapeutic state, circulatory insufficiency in the common cochlear artery might have occurred as a result of arteriosclerosis secondary to hyperlipidemia, causing changes in the stria vascularis that resulted in hearing loss.

The cause of sudden deafness is not uniform, and we can also consider other causes of hearing loss in our patients. The hearing loss at the higher frequencies showed only a slight response to therapy. Further, the hearing improvement at the lower frequencies was not noted in all patients. These findings suggest the participation of other organic changes, such as degeneration in the organ of Corti, in the development of hearing loss. We consider that the therapy for hyperlipidemia would affect exclusively hearing loss attributable to reversible changes in the stria vascularis.

Among the several hypotheses as to the cause of sudden deafness, in addition to the circulatory disturbance hypothesis described above, viral infection is also implicated frequently. The present study suggested the possibility of arteriosclerosis and circulatory disturbance secondary to hypercholesterolemia as a cause of hearing loss in patients with sudden deafness.

Although hyperlipidemia is usually treated by internists, we as otolaryngologists were able to manage the hyperlipidemia in our patients. When otolaryngologists treat hyperlipidemia associated with chronic-phase sudden deafness, they should keep several points in mind. The audiogram and blood level of total cholesterol must be checked on the same day for observation of the relationship between hearing level and total cholesterol level. Diet therapy, the treatment of first choice for hyperlipidemia, should be tried for about a month, but administration of antilipemic agents may be necessary when diet therapy is not successful. Antilipemic agents, however, have side effects. For example, pravastatin has an adverse effect on muscle systems, which can be monitored by measuring the creatine phosphokinase level. None of our patients had a total blood cholesterol level exceeding 275 mg/dL, but in patients whose level is 300 mg/dL or greater, there is a possibility of familial disease, and it is advisable to consult with an internist.

If these points are kept in mind, otolaryngologists can relatively safely treat patients with hyperlipidemia associated with sudden deafness. Although this study focused on patients with chronic sudden deafness accompanied by hyperlipidemia, this treatment may also be effective for patients with other types of sensorineural hearing loss accompanied by hyperlipidemia and circulatory disorders.

If we had performed randomized clinical trials or had examined historical controls in this study, our conclusions would be more certain. In our opinion, chronic-phase sudden deafness rarely recovers and the hearing levels do not change. In our study, we treated all patients with chronic-phase sudden deafness and hyperlipidemia. Thus, we did not have historical controls who had both chronic-phase sudden deafness and untreated hyperlipidemia. This treatment method was not administered for all patients with chronic-phase sudden deafness.

SUMMARY

We investigated the effects of therapy for hyperlipidemia on the hearing loss in 12 patients with unilateral sudden deafness with duration longer than 1 month and associated hyperlipidemia. The mean hearing level improved significantly at each of 125, 250, 500, and 2,000 Hz after therapy. It is suggested that hearing tends to improve with therapy for hyperlipidemia in patients with chronic-phase sudden deafness presumed to be irreversible.

REFERENCES

1. Schuknecht HF. Pathology of the ear. Cambridge, Mass: Harvard University Press, 1974.

2. Gussen R. Sudden deafness of vascular origin: a human temporal bone study. Ann Otol Rhinol Laryngol 1976;85:94-100.

3. De Kleyn A. Sudden complete or partial loss of function of the octavus-system in apparently normal persons. Acta Otolaryngol (Stockh) 1944;32:407-29.

4. Rosen S, Plester D, El-Mofty A, Rosen HV. Relation of hearing loss to cardiovascular disease. Trans Am Acad Ophthalmol Otolaryngol 1964;68:433-44.

5. Spencer JT. Hyperlipoproteinemias in the etiology of inner ear disease. Laryngoscope 1973;83:639-78.

6. Saito T. An experimental study on auditory dysfunction associated with hyperlipoproteinemia. J Otolaryngol Jpn 1985; 88:319-29.

7. Yamane H, Nakai Y, Chang KC, Harada H, Sato K. The inner ear of guinea pigs with experimental hyperlipidemia. Pract Otol (Kyoto) 1983;76:1611-23.

8. Nabeya D. A study in the comparative anatomy of the blood-vascular system of the internal ear in mammalia and in homo (Japanese). Acta Sch Med Univ Imp Kyoto 1923;6:1-132.

Ann Otol Rhinol Laryngol 110:2001

EFFECTS OF EXPOSING THE OPENED ENDOLYMPHATIC SAC TO LARGE DOSES OF STEROIDS TO TREAT INTRACTABLE MENIERE'S DISEASE

TADASHI KITAHARA, MD, PHD
NORIAKI TAKEDA, MD, PHD
YASUO MISHIRO, MD
TAKANORI SAIKA, MD, PHD
MUNEHISA FUKUSHIMA, MD
SHIN-ICHI OKUMURA, MD, PHD
TAKESHI KUBO, MD, PHD
OSAKA, JAPAN

To enhance the effect of treatment for intractable Meniere's disease, we exposed the opened endolymphatic sac to high concentrations of steroids. This technique — endolymphatic sac drainage and steroid instillation surgery — involves the application of a mass of prednisolone followed by absorbable gelatin sponges soaked in a high concentration of dexamethasone into a sac lumen opened and expanded with a bundle of absorbable gelatin film. These sponges are also placed around the sac and coated with biochemical adhesive so that the medicine is slowly delivered into the sac over a prolonged period of time by means of a natural sustained-release vehicle. The short-term results (6 to 14 months) in 12 patients with Meniere's disease, including those in stage IV, treated by the above techniques showed that definitive spells were completely controlled in all cases. Hearing was improved, and annoyance due to tinnitus was decreased in all cases except one.

KEY WORDS — endolymphatic sac surgery, gelatin film, Meniere's disease, steroid, steroid instillation surgery.

INTRODUCTION

Meniere's disease is usually associated with a small, displaced, malfunctioning endolymphatic sac[1] and is triggered by an immune, metabolic, infectious, traumatic, or other insult to the inner ear.[2] One of the main causes of endolymphatic hydrops is thought to be an immune-mediated response in various organs in the inner ear, such as the endolymphatic sac, stria vascularis, or spiral ligament.[3-6] Thus, systemic administration and/or local perfusion in the middle ear of corticosteroids has been adopted[7,8] for patients with Meniere's disease. These treatments have been revealed to give some relief from vertigo, but little improvement of hearing.

To enhance the efficacy of treating the above symptoms of Meniere's disease, a new technique — endolymphatic sac drainage and steroid instillation surgery (EDSS) — was designed. This technique is planned to expose the diseased inner ear to large doses of steroids via the opened endolymphatic sac. In the present study, we describe the technique of EDSS and its effect on intractable Meniere's disease.

MATERIALS AND METHODS

We performed EDSS in 12 patients with a definitive diagnosis of Meniere's disease[9] (Table 1). There were 5 women and 7 men, ranging in age from 24 to 67 years (mean, 50.8 years). All of these patients had intractable Meniere's disease for which various medical management strategies had failed. The average duration of disease before surgical treatment was 5.0 years (range, 1.0 to 9.5 years). The average number of definitive spells of vertigo per month during the 6 months before surgery was 2.0 (range, 0.5 to 4.0 spells). The numbers of patients in stages I, II, III, and IV[10] were 0, 1, 6, and 5, respectively.

The technical details of EDSS are as follows. A simple mastoidectomy was performed, clearly exposing the endolymphatic sac in the area between the sigmoid sinus and the inferior margin of the posterior semicircular canal. The sac was opened with an L-shaped (right ear) or backward L–shaped (left ear) incision made along the posterior and distal margins of the lateral wall. Then the sac was filled with a 20-mg mass of prednisolone. While the mass dissolved in the sac, we prepared a bundle of absorbable gelatin films (4 × 20 × 0.7 mm, 5 sheets) with fan-shaped and stick-shaped ends. These films were tied to each other with biochemical adhesive (human thrombin combined with human fibrinogen) only at the stick-shaped end in the same manner as for sac-expanding surgery.[11] Then, the fan-shaped end was inserted into the sac, and small pieces of absorbable gelatin sponge

---

From the Department of Otolaryngology, Osaka Rosai Hospital, Sakai (Kitahara, Okumura), and the Department of Otolaryngology, Osaka University, Medical School, Suita (Takeda, Mishiro, Saika, Fukushima, Kubo), Osaka, Japan. This study was supported in part by a Health Science Research Grant for Specific Disease of the Ministry for Health and Welfare, Japan (1999-2001).

CORRESPONDENCE — Tadashi Kitahara, MD, PhD, Dept of Otolaryngology, Osaka Rosai Hospital, 1179-3 Nagasonecho, Sakai, Osaka 591-8025, Japan.

TABLE 1. PREOPERATIVE PATIENT DATA

Patient No.	*Age (y)*	*Sex*	*Duration of Disease (y)*[a]	*Frequency of Vertigo*[b]	*Hearing Level (dB)*[c]	*Tinnitus Score*[d]
1	54	F	1.4	0.8	45.0	5
2	63	F	4.7	0.8	56.3	4
3	43	M	2.5	0.5	83.8	4
4	47	F	6.5	1.5	47.5	4
5	39	F	8.2	3.3	70.0	4
6	67	M	9.5	2.0	81.3	2
7	55	M	9.3	0.5	50.0	2
8	24	F	3.5	4.0	77.5	4
9	47	M	5.0	2.5	80.0	4
10	53	M	1.0	4.0	40.0	3
11	55	M	5.0	1.5	77.5	4
12	61	M	4.0	2.0	58.8	4

[a] Duration of disease before surgery.

[b] Average number of definitive spells per month for 6 months before surgery.

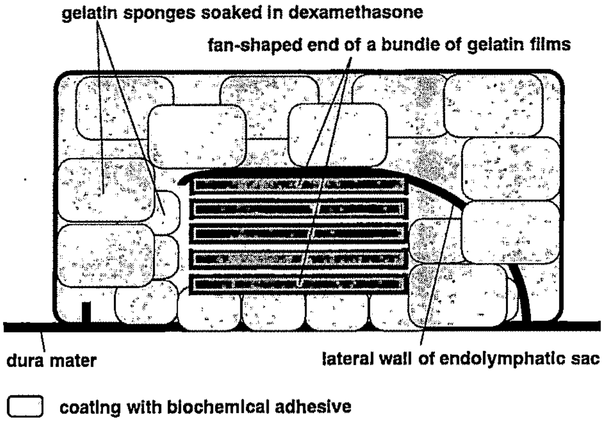
[c] Poorest hearing level obtained in 6 months before surgery.

[d] Score (see text) of annoyance caused by tinnitus on average detected at remission stages during 6 months before surgery.

soaked in a high concentration of dexamethasone (32 mg/4 mL) were placed inside and outside the sac lumen expanded with the bundle. The sponges containing dexamethasone placed outside the sac were coated with the adhesive so that dexamethasone was slowly delivered into the sac over a long period of time by means of a natural sustained-release vehicle (see Figure). The stick-shaped end of the gelatin film bundle extending out of the sac was fixed to the front edge of the mastoid cavity with the same adhesive, so that the incision into the sac was expanded for an adequate period of time after surgery. The mastoid cavity was filled with relatively large pieces of absorbable gelatin sponge dipped in antibiotic solution, and the wound was closed with skin sutures.

RESULTS

The short-term results (6 to 14 months) in 12 patients with intractable Meniere's disease who underwent EDSS are shown in Table 2. Definitive spells were completely controlled in all cases. Hearing was improved (gain within the speech frequency pure tone average, ≥10 dB) in all cases except one, in comparison with the poorest hearing levels obtained in the 6 months before surgery. Immediately after surgery, it was common for hearing to worsen, but then it continuously improved for approximately 2 to 8 weeks or longer. The worsened hearing in the period directly after surgery was excluded from calculation. Because the hearing loss in Meniere's disease is known to fluctuate, the widths of hearing fluctuation in the 6 months before surgery are also shown in Table 2; 10 of the 12 patients had a postoperative hearing gain greater than the width of the preoperative hearing fluctuation. Tinnitus was evaluated on a 5-point scale of annoyance according to the criteria proposed by the Tinnitus Study Group Japan in 1993 (1 — tinnitus is usually ignored; 2 — tinnitus is ignored while working; 3 — tinnitus is sometimes irritating while working; 4 — tinnitus is irritating, but causes little or no interference with work; 5 — tinnitus is irritating and renders me unable to perform any work). In 6 cases, the annoyance caused by tinnitus had disappeared (average score decreased to 1) in the remission periods after surgery, as compared to the remission periods during the 6 months before surgery. In 5 cases, for the same comparison, the annoyance had decreased (average score decreased by 1 or more grades).


gelatin sponges soaked in dexamethasone
fan-shaped end of a bundle of gelatin films
dura mater
lateral wall of endolymphatic sac
▭ coating with biochemical adhesive

Schematic representation of endolymphatic sac drainage and steroid instillation surgery. Cross section of endolymphatic sac and fan-shaped end of bundle of gelatin films is shown. Absorbable gelatin sponges containing high concentration of steroids were placed around sac and then coated with biochemical adhesive to provide sustained release.

DISCUSSION

There have been a number of articles in the literature on Meniere's disease that affirm the clinical value of systemic and middle ear application of steroids[7,8] and various types of endolymphatic sac surgery.[11-16] Almost all of the articles report that vertigo was con-

TABLE 2. SHORT-TERM RESULTS

Patient No.	*Follow-up (mo)*	*Vertigo*[a]	*Preoperative Hearing Fluctuation (dB)*[b]	*Improvement in Hearing Level (dB)*[c]	*Decrease in Tinnitus Score*[d]
1	14	0	11.2	16.2	3
2	13	0	23.8	18.8	3
3	11	0	16.3	3.8	3
4	9	0	5.0	22.5	3
5	8	0	15.0	16.2	1
6	8	0	15.0	21.3	0
7	7	0	17.5	33.7	1
8	7	0	20.0	37.5	3
9	6	0	7.5	11.2	2
10	6	0	13.8	15.0	2
11	6	0	2.5	10.0	2
12	6	0	10.0	13.8	2

[a] Number of postoperative definitive spells.
[b] Preoperative poorest hearing level minus preoperative best hearing level.
[c] Preoperative poorest hearing level minus postoperative hearing level.
[d] Preoperative tinnitus score (see text) minus postoperative tinnitus score.

trolled in 77% to 100%, but that hearing was improved in fewer than 30% of cases. As for cases in stage IV, few patients could expect a significant hearing improvement. Results on tinnitus are not clearly described in most reports. The short-term results (6 to 14 months) of EDSS in patients with intractable Meniere's disease, including stage IV, were excellent. Definitive spells were completely controlled in all cases. Hearing was improved, and annoyance from tinnitus was decreased in all cases except one. Although our success rate with EDSS is admittedly preliminary, the effects of EDSS on inner ear symptoms, especially on hearing and tinnitus, including cases in stage IV, are considered unique. Because the only significant variable distinguishing EDSS from other techniques is the application of steroids into the opened and expanded endolymphatic sac, it could be concluded that the remarkable improvement in symptoms is attributable to the effects of large doses of steroids instilled into the sac.

The mechanisms of EDSS's effects on the labyrinthine functions are thought to be multiple steroid effects: anti-inflammatory, anti-edematic, and vasoactive.[17,18] Because recent clinical and laboratory evidence has increasingly indicated that Meniere's disease is an immune-mediated disease of inner ear structures such as the endolymphatic sac, stria vascularis, and spiral ligament,[3-6] one of the most likely mechanisms of these effects may be anti-immunoreactive.[19] Steroids directly instilled into the endolymphatic cavity may be more effective on the diseased inner ear organs than those applied via any other route. However, further basic studies are needed to determine how much of the steroid instilled into the sac reaches the vestibule and cochlea through the vestibular aqueduct. It is not too much to say that drainage of endolymphatic sac fluid into the mastoid cavity could have also contributed to the satisfactory results of EDSS.

In EDSS, absorbable gelatin sponges containing high concentrations of steroids, placed around the endolymphatic sac, are coated with biochemical adhesive so that the medicine will be delivered into the sac for a prolonged period of time. However, the durability of the improvements in inner ear functions achieved by steroids instilled into the sac remains unknown. The most controversial aspect of sac surgery is the recurrence of symptoms in the long term.[20-23] Therefore, it is difficult to present a definitive evaluation of the treatment effects of EDSS without sufficient materials and long-term observation. However, the improvements in hearing and tinnitus, as well as vertigo, in patients with Meniere's disease, including those in stage IV, exceeded our expectations. The short-term but unique results reported in this article encourage us to treat patients with intractable or progressive Meniere's disease with this technique. Further studies on EDSS will be reported in later communications.

REFERENCES

1. Shambaugh GE Jr, Clemis JD, Arenberg IK. Endolymphatic duct and sac in Meniere's disease. I. Surgical and histopathologic observations. Arch Otolaryngol 1969;89:816-25.

2. Gutierrez F, Moreno PM, Sainz M. Relationship between immune complex and total hemolytic complement in endolymphatic hydrops. Laryngoscope 1994;104:1495-8.

3. Brooks GB. Circulating immune complexes in Meniere's disease. Arch Otolaryngol Head Neck Surg 1986;112:536-40.

4. Hughes GB, Kinney SE, Barna BP, Calabrese LH. Autoimmune reactivity in Meniere's disease: a preliminary report.

Laryngoscope 1983;93:410-7.

5. Veldman JE, Roord JJ, O'Connor AF, Shea JJ. Autoimmunity and inner ear disorders. Laryngoscope 1984;94:501-7.

6. Yamanobe S, Harris JP. Inner ear–specific autoantibodies. Laryngoscope 1993;103:319-25.

7. Itoh A, Sakata E. Treatment of vestibular disorders. Acta Otolaryngol Suppl (Stockh) 1991(suppl 481):617-23.

8. Shea JJ, Ge X. Dexamethasone perfusion of the labyrinth plus intravenous dexamethasone for Meniere's disease. Otolaryngol Clin North Am 1996;29:353-8.

9. Watanabe I. Meniere's disease. ORL J Otorhinolaryngol Relat Spec 1980;42:20-45.

10. Committee on Hearing and Equilibrium. Committee on Hearing and Equilibrium guidelines for diagnosis and evaluation of therapy in Meniere's disease. Otolaryngol Head Neck Surg 1995;113:181-5.

11. Kitahara M, Goto E. Sac expanding surgery for Meniere's disease. In: Proceedings of the 4th International Symposium on Meniere's Disease. The Hague, the Netherlands: Kugler Publications (in press).

12. Arenberg IK. Results of endolymphatic sac to mastoid shunt surgery for Meniere's disease. Am J Otol 1987;8:335-44.

13. Huang TS, Lin CC. Endolymphatic sac ballooning surgery for Meniere's disease. Ann Otol Rhinol Laryngol 1994;103: 389-94.

14. Moffat DA. Endolymphatic sac surgery: analysis of 100 operations. Clin Otolaryngol 1994;19:261-6.

15. Paparella MM, Sajjadi H. Endolymphatic sac enhancement. Otolaryngol Clin North Am 1994;27:381-402.

16. Pensak ML, Friedman RA. The role of endolymphatic mastoid shunt surgery in the managed care era. Am J Otol 1998; 19:337-40.

17. Lohuis PJ, ten Cate WJ, Patter KE, Rarey KE. Modulation of the rat stria vascularis in the absence of circulating adrenocorticosteroids. Acta Otolaryngol (Stockh) 1990;110:348-56.

18. Pitovski DZ, Drescher MJ, Kerr TP, Drescher DG. Aldosterone mediates an increase in [^{3}H]ouabain binding at Na+,K(+)-ATPase sites in the mammalian inner ear. Brain Res 1993;601:273-8.

19. Rarey KE, Lohuis PJ, ten Cate WJ. Response of the stria vascularis to corticosteroids. Laryngoscope 1991;101:1081-4.

20. Goin DW, Mischke RE, Esses BA, Young D, Priest EA, Whitmoyer-Goin V. Hearing results from endolymphatic sac surgery. Am J Otol 1992;13:393-7.

21. Jackson CG, Dickins JRE, McMenomey SO, et al. Endolymphatic system shunting: a long-term profile of the Denver Inner Ear Shunt. Am J Otol 1996;17:85-8.

22. Quaranta A, Onofri M, Sallustio V, Iurato S. Comparison of long-term hearing results after vestibular neurectomy, endolymphatic mastoid shunt and medical therapy. Am J Otol 1997;18:444-8.

23. Silverstein H. Natural history vs. surgery for Meniere's disease. Otolaryngol Head Neck Surg 1989;100:6-16.

≈

INTERNATIONAL HEARING AID CONFERENCE

International Hearing Aid Conference VI. Novel Processing and Fitting Strategies will be held in Iowa City, Iowa, June 7-9, 2001. For information, see web site www.medicine.uiowa.edu/otolaryngology/news/news, or contact Rich Tyler by telephone (319-356-2471) or e-mail (rich-tyler@uiowa.edu).

Ann Otol Rhinol Laryngol 110:2001

INNER EAR FLUID VOLUMES AND THE RESOLVING POWER OF MAGNETIC RESONANCE IMAGING: CAN IT DIFFERENTIATE ENDOLYMPHATIC STRUCTURES?

RICHARD A. BUCKINGHAM, MD GALDINO E. VALVASSORI, MD
CHICAGO, ILLINOIS

Magnetic resonance imaging (MRI) can accurately recognize minute volumes as small as 1 mm^3. The volumes of the utricle and saccule of the inner ear are within the resolving power of MRI, but these structures cannot be recognized because the endolymph and perilymph signals are identical. To clarify the interpretation and description of inner ear structures on MRI, we measured and calculated the volumes of the perilymphatic and endolymphatic spaces of the human ear. We found the total volume of the bony labyrinth to be approximately 192.5 mm^3 (endolymph, 34.0 mm^3; perilymph, 158.5 mm^3).

KEY WORDS — inner ear volume, labyrinth volume, magnetic resonance imaging.

INTRODUCTION

With present-day techniques, magnetic resonance imaging (MRI) is capable of distinguishing volumes as small as the different components of the bony labyrinth. We became interested in knowing the actual volumes of the perilymphatic and endolymphatic spaces of the inner ear structures, and whether it is possible to recognize such structures as the saccule, utricle, and membranous semicircular canals on MRI. In a search of the literature, including anatomy textbooks,[1-9] we found only 1 report[1] with more than isolated, incomplete data on the volumes of these structures.

Chemically, the inner ear fluids differ, in that endolymph has a higher concentration of potassium, whereas perilymph has little potassium, but a relatively high sodium concentration. In addition, the concentration of protein in perilymph is as much as 6 times higher than that in endolymph.[10] However, the fact remains that endolymph and perilymph produce an identical MRI signal, no matter which sequence is used.

Replacement of the normal labyrinthine fluids by blood, pus, fibrous tissue, bone, or tumors, such as schwannomas and lipomas, alters the MRI signal and can be detected. Changes in the permeability of the blood vessels of the labyrinth due to a viral infection also alter the MRI signal seen when contrast is injected.

In a review of the MRI literature, we have noted an ambivalence and confusion of terminology when the finer points of labyrinthine anatomy are described. The terms membranous labyrinth, bony labyrinth, inner ear, and endolymphatic and perilymphatic spaces are sometimes used as though they were interchangeable.[11-17]

This somewhat indiscriminate use of morphological terms led us to study the anatomy of the inner ear and the volume of its contents, to determine whether it is possible to differentiate structures that contain endolymph from those that contain perilymph, and to visualize the membranous labyrinth with present-day MRI resolution.

A brief review of the inner ear anatomy might be helpful in clarifying the terminology. The bony labyrinth spaces, which lie within the temporal bone, are filled with perilymph and endolymph. The smaller membranous labyrinth, which contains endolymph, lies within the bony labyrinth spaces and more or less floats within the perilymph (Fig 1[18]). *Gray's Anatomy*[2(p1380)] defines the membranous labyrinth as a "continuous system of ducts contained within the osseous labyrinth." Perilymph is in direct communication with the subarachnoid space via the cochlear aqueduct. The endolymph-containing spaces have no connection with extralabyrinthine spaces and terminate in the closed endolymphatic sac, which lies within the dura of the posterior cranial fossa.

METHODS

To determine the labyrinth volume, we studied the literature, queried other workers in the field, and calculated the volume by using the dimensions from anatomy texts. In addition, we measured the volume of water displaced when we immersed a plastic cast

---

From the Department of Otolaryngology–Head and Neck Surgery, Resurrection Hospital (Buckingham), and the Departments of Radiology and Otolaryngology–Head and Neck Surgery, University of Illinois at Chicago, College of Medicine (Valvassori), Chicago, Illinois.

CORRESPONDENCE — Richard A. Buckingham, MD, 145 S Northwest Hwy, Park Ridge, IL 60068.

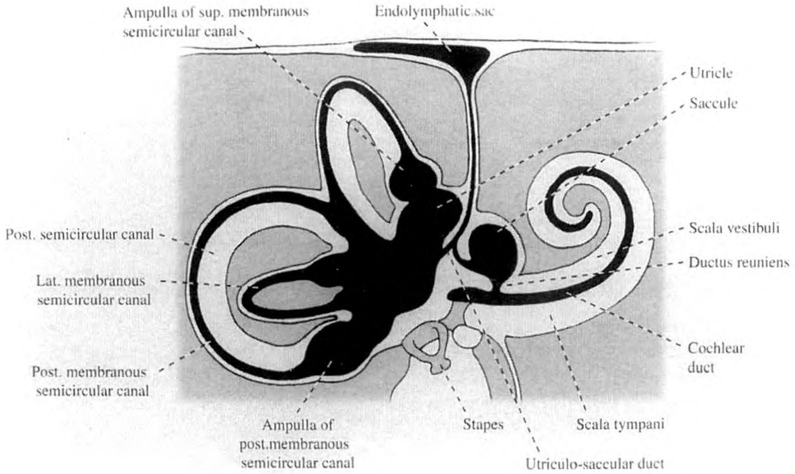

Ampulla of sup. membranous semicircular canal
Endolymphatic sac
Utricle
Saccule
Post. semicircular canal
Lat. membranous semicircular canal
Post. membranous semicircular canal
Scala vestibuli
Ductus reuniens
Cochlear duct
Ampulla of post.membranous semicircular canal
Stapes
Scala tympani
Utriculo-saccular duct

Fig 1. Schematic drawing of inner ear. (Modified from Sobotta.[18]) Endolymphatic structures are depicted in black. Gray areas represent temporal bone and surrounding bony labyrinth. Perilymph, which surrounds and bathes endolymph structures, is white.

of the entire labyrinth into a 3-mL syringe.

We searched the literature and the anatomy texts at the University of Illinois medical library and the Internet for information concerning the volume of perilymph and endolymph.[1-16] We found no figures for the volume of the bony labyrinth in anatomy texts, but obtained dimensions of different parts of the bony and membranous labyrinth from various reports. For instance, in the 1995 edition of *Gray's Anatomy*,[2] the dimensions of the vestibule are given as 5 × 3 × 3 mm; the 3 bony semicircular canals are said to be 12 to 22 mm long; and the 3 ampullae of the semicircular canals are said to be approximately 1.6 mm in diameter. The cochlea is a cone-shaped structure approximately 9 mm in diameter at the base and 4 to 5 mm high.

In 1967, Igarashi[9] reported dimensions of several labyrinth structures. He found the lumens of the membranous semicircular canals to be oval, with a diameter of 0.44 × 0.25 mm, and he found the diameter of the oval bony semicircular canals to be 1.4 × 1.0 mm. He reported the diameter of the ampullae to be 2.07 mm. He gave no estimate of the volumes of the entire labyrinth. In 1983, Igarashi et al[7] reported the volumes of the utricle and saccule to be 8.1 and 2.0 mm^3, respectively. In 1986, Igarashi et al[1] reported on volumes of the endolymphatic and perilymphatic spaces in human temporal bones. They used a method of studying histologic serial sections of the human temporal bone and determining the volumes with a computer. They found the total volume of the labyrinth to be 204.5 mm^3: 38.1 mm^3 for endolymph and 164.4 mm^3 for perilymph. These authors found the total cochlear volume to be 83.6 mm^3: 75.24 mm^3 for perilymph and 8.36 mm^3 for endolymph.

Dr Isamu Sando, at the Elizabeth McCullough Knowles Otopathology Laboratory in Pittsburgh,

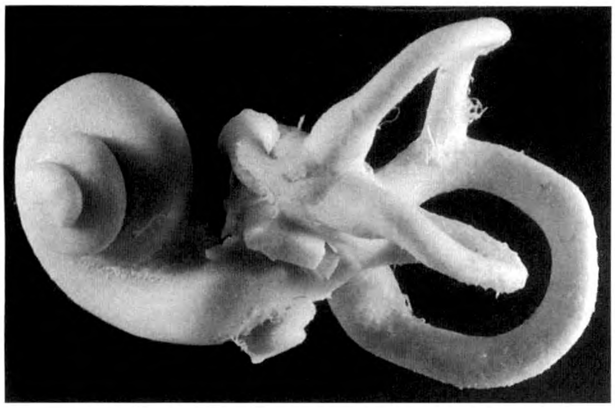


Fig 2. Photograph of Wilbrand labyrinthine cast.

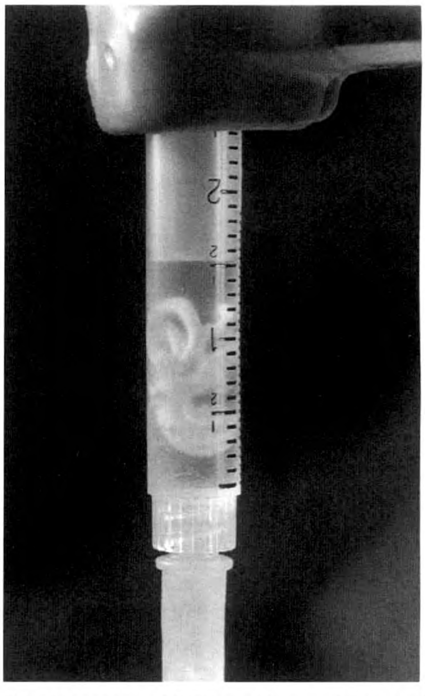


Fig 3. Displacement of Wilbrand's plastic cast of labyrinth. Pliant mold of inner ear fits into barrel of 3-mL syringe, and water displacement is measured with Zeiss microscope to be 0.2 mL (200 mm^3).

GEOMETRIC CALCULATIONS OF INNER EAR STRUCTURES

Structure	*Shape*	*Dimensions (mm)*	*Volume (mm^3)*
Bony labyrinth and perilymphatic and endolymphatic spaces			
Semicircular canals	Cylinder	1.2 × 16 × 3	54.2
Ampullae	Sphere	2.1 × 3	14.5
Cochlea	Cone	8 × 5	83.8
Vestibule	Irregular cylinder	2.8 × 6.5	40
		Total	192.5
Membranous endolymphatic labyrinth spaces			
Semicircular canals	Cylinder	0.34 × 16.0 × 3	4.35
Ampullae	Sphere	2.0 × 3	12.5
Cochlear duct	Cylindrical triangle	0.5 × 0.8 × 36.0 × 0.5	7.2
Saccule	Hemisphere	2.0	2.1
Utricle	Irregular oval	1.35 × 5.5 × 0.785	7.9
		Total	34.05

Water displacement of bony labyrinth was 200 mm^3.

Ratio of endolymph to perilymph is 34/158.5 or 21.5%; total volume of perilymphatic space and endolymphatic space is 192.5 mm^3; volume of endolymphatic space is 34.0 mm^3; thus, volume of perilymphatic space is 192.5 – 34.0 = 158.5 mm^3.

Calculations for membranous labyrinth: each of 3 membranous canals has average diameter (D) of 0.34 mm and length of 16 mm by formula: area of circle = 0.785 D^2; 0.34^2 × 0.785 × 16 × 3 = 4.35 mm^3.

Ampulla is sphere 2 mm in diameter. Volume of sphere = 0.524 D^3, hence, 2 × 2 × 2 × 0.524 × 3 = 12.5 mm^3.

Cochlear duct is roughly triangular column: (0.5 × 0.8)/2 × 36 = 7.2 mm^3.

Saccule is hemisphere 2 mm in diameter. Volume of sphere = 0.524 D^3, hence, 2 × 2 × 2 × 0.524 × .5 = 2.1 mm^3.

Membranous utricle is irregular ovoid sac averaging 1.35 mm in diameter and 5.5 mm in length: 1.35 × 1.35 × 0.785 × 5.5 = 7.9 mm^3.

Pennsylvania, in a personal communication (1996), reported that he had measured the volume of the inner ear fluid spaces using serial histologic sections of the temporal bone of a 5-year-old boy by computer-aided reconstruction. The total volume was 188 mm^3: 46 mm^3 for endolymph and 142 mm^3 for perilymph. He mentioned that his figures may be on the low side because of shrinkage artifact of approximately 10% with the temporal bone histologic process.

To determine the volume of both the endolymphatic and perilymphatic spaces, we used a pliant plastic mold of the inner ear supplied to us by Professor H. F. Wilbrand, Department of Diagnostic Radiology, University of Uppsala, Sweden. Wilbrand and Rauschning[19] developed a method of pumping liquid plastic into the lumens of dry temporal bones. When the plastic hardens, they leach out the calcium, and the result is a perfect cast of the spaces and canals of the labyrinth that is flexible and malleable, but not compressible.

Thus, we obtained a flexible cast of the vestibule, semicircular canals, and cochlea (Fig 2). To measure the volume of the cast, we placed it into a water-filled 3-mL syringe that was mounted on a ring stand as illustrated in Fig 3. With a Zeiss microscope, it was easy to determine the displacement of water by the labyrinth cast. Ten measurements were made. We found the volume to be 0.2 mL, which is equal to 200 mm^3 or 200 μL. This method is accurate only to 0.01 mL (Fig 3). Thus, the total volume of the bony labyrinth is about 4 drops of water when water is dropped from a standard dropper of 20 drops per milliliter. As we shall see below, the ratio of endolymph to perilymph is approximately 1:5, and thus the total volume of endolymph in the human ear would be less than 1 standard drop.

Using measurements from the plastic mold, histologic cross sections, and macrosections, as well as average figures given in various anatomy books and published articles,[1-9] we calculated the volume of the components of the bony labyrinth. The bony labyrinth is divided into 3 semicircular canals, 3 ampullae, a vestibule, and a cochlea (Fig 1). These figures are summarized in the Table and coincide with our displacement findings of the plastic labyrinth. These calculations serve as a cross-check to our water displacement method and give us some degree of confidence as we calculate the volume of the endolymph.

To determine the volume of the endolymphatic spaces, we used histologic sections, macrosections, and measurements from several other sources.[1-9] We divided the endolymphatic spaces into 3 semicircular canals and ampullae, 1 utricle, 1 saccule, and a cochlear duct. The results of these calculations are included in the Table. We compared our results with those of Igarashi et al[1,7,9] and Takagi and Sando.[6] We found our figures for the membranous labyrinth spaces to be approximately 20% smaller than those reported by Sando (personal communication, 1996), but close to those of Igarashi et al.[1]

The mathematical method using geometric formulae and measurements from anatomy books and histologic sections can provide only approximate figures. We are aware of individual variations in the dimensions of the labyrinth that would tend to alter our values somewhat, but not essentially. We used these methods as the best practical approach to determining labyrinth volumes.

RESULTS

Our results are summarized in the Table. The volume of the bony labyrinth calculated from various anatomic dimensions — 192.5 mm^3 (0.192 mL) — is close to the value of our one plastic inner ear cast, which displaced 200 mm^3 of water, and to the values reported by Takagi and Sando[6] (188 mm^3) and Igarashi[9] (204.5 mm^3).

We calculated the volume of endolymph within the three semicircular canals, including the ampullae, to be approximately 17 mm^3 (0.17 mL), and the volume of endolymph within the cochlear duct to be approximately 7.2 mm^3 (0.072 mL). The normal utricle has a volume of 7.9 mm^3 (0.079 mL), and the saccule has a volume of 2.1 mm^3 (0.021 mL). These last two structures occupy one fourth of the vestibular space. The total endolymphatic space is 34.0 mm^3. Thus, the volume of the perilymphatic space is approximately 192.5 mm^3 minus 34.0 mm^3, or 158.5 mm^3.

DISCUSSION

The normal endolymphatic sac contains only a minute amount of liquid, sufficient to make the lumen of the sac moist. In other words, there is no measurable amount of endolymph in the normal endolymphatic sac. The normal vestibular aqueduct contains a minute endolymphatic duct surrounded by connective tissue. This explains why in T2 fast spin images the vestibular aqueduct has a signal lower than that of the other inner ear structures, because connective tissue is less bright than fluid.

The walls of the membranous labyrinth are too thin to be recognized, but the macula of the utricle can occasionally be seen, on end, in coronal images. This appears as a fine, thin band of low signal intensity within the upper portion of the vestibule in coronal images, as demonstrated by the exhibit of Mafee at the meeting of the Radiological Society of North America in Chicago in 1996.

Although the resolution of present-day MRI systems allows recognition of volumes smaller than those of the utricle and saccule, endolymph cannot be differentiated from perilymph, not because of volume averaging problems, but simply because MRI in its present state cannot differentiate the two fluids nor the labyrinth membranous walls.

CONCLUSIONS

To judge the advantages and limitations of MRI as its resolution has approached the microscopic dimensions of the labyrinth structures, we measured and calculated the volumes of the perilymphatic and endolymphatic spaces of the labyrinth, because only fragmentary data are available in the literature. Our calculations are in agreement with the reported results of Takagi and Sando[6] and Igarashi.[9] We look forward to continually improving techniques and to the time when, hopefully, we will be able to visualize these two fluid spaces in the labyrinth.

REFERENCES

1. Igarashi M, Ohashi K, Ishii M. Morphometric comparison of endolymphatic and perilymphatic spaces in human temporal bones. Acta Otolaryngol (Stockh) 1986;101:161-4.

2. Williams PL, ed. Gray's anatomy: the anatomic basis of medicine and surgery. New York, NY: Churchill Livingstone, 1995.

3. Anson BJ, Donaldson JA. Surgical anatomy of the temporal bone. Philadelphia, Pa: Lippincott-Raven, 1992.

4. Curthoys IS, Blanks RHI, Markham CH. Semicircular canal radii of curvature (R) in cat, guinea pig and man. J Morphol 1977;151:1-16.

5. Curthoys IS, Markham CH, Curthoys EJ. Semicircular duct and ampulla dimensions in cat, guinea pig and man. J Morphol 1977;151:17-34.

6. Takagi A, Sando I. Computer-aided three-dimensional reconstruction: a method of measuring temporal bone structures including the length of the cochlea. Ann Otol Rhinol Laryngol 1989;98:515-22.

7. Igarashi M, O-Uchi T, Isago H, Wright WK. Utricular and saccular volumetry in human temporal bones. Acta Otolaryngol (Stockh) 1983;95:75-80.

8. Curthoys IS, Oman CM. Dimensions of the horizontal semicircular duct, ampulla and utricle in the human. Acta Otolaryngol (Stockh) 1987;103:254-61.

9. Igarashi M. Dimensional study of vestibular apparatus. Laryngoscope 1967;77:1806-17.

10. Wangemann P, Schacht J. Homeostatic mechanisms in the cochlea. In: Dallos P, Popper AN, Fay RR, eds. The cochlea. Berlin, Germany: Springer, 1996:150-3.

11. Brogan M, Chakeres DW, Schmalbrock P. High-resolution 3DFT MR imaging of the endolymphatic duct and soft tissues of the otic capsule. AJNR Am J Neuroradiol 1991;12:1-11.

12. Seltzer S, Mark AS. Contrast enhancement of the labyrinth on MR scans in patients with sudden hearing loss and vertigo: evidence of labyrinthine diseases. AJNR Am J Neuroradiol 1991;12:13-6.

13. Casselman JW, Kuhweide R, Deimling M, Ampe W, De-

haene I, Meeus L. Constructive interference in steady state–3DFT MR imaging of the inner ear and cerebellopontine angle. AJNR Am J Neuroradiol 1993;14:47-57.

14. Welling DB, Clarkson MW, Miles BA, et al. Submillimeter magnetic resonance imaging of the temporal bone in Meniere's disease. Laryngoscope 1996;106:1359-64.

15. Schmalbrock P, Dailiana T, Chakeres DW, et al. Submillimeter-resolution MR of the endolymphatic sac in healthy subjects and patients with Meniere disease. AJNR Am J Neuroradiol 1996;17:1707-16.

16. Casselman JW, Kuhweide R, Ampe W, Meeus L, Steyaert L. Pathology of the membranous labyrinth: comparison of T1- and T2-weighted and gadolinium-enhanced spin-echo and 3DFT-CISS imaging. AJNR Am J Neuroradiol 1993;14:59-69.

17. Dimopoulos P, Muren C. Anatomic variations of the cochlea and relations to other temporal bone structures. Acta Radiol 1990;31:439-44.

18. Sobotta J. Atlas der descriptiven Anatomie. Teil 3. Berlin: Urban und Schwartzenberg, 1946.

19. Wilbrand H, Rauschning W. Investigation of temporal bone anatomy by plastic molding and cryomicrotomy. Acta Radiol Diagn 1986;27:389-94.

≈

EIGHTH SYMPOSIUM ON COCHLEAR IMPLANTS IN CHILDREN

The Eighth Symposium on Cochlear Implants in Children will be held in Los Angeles, California, February 28 to March 3, 2001. For information, visit the web site www.hei.org/ci2001; telephone Caroline Sakai at (213) 353-7001; or write to House Ear Institute/CI2001, 2100 W Third St, Los Angeles, CA 90057.

≈

NEW FRONTIERS IN THE AMELIORATION OF HEARING LOSS

The international conference New Frontiers in the Amelioration of Hearing Loss will be held in St Louis, Missouri, March 22-25, 2001. For information, contact Sarah Uffman, Central Institute for the Deaf, Department of Research, 4560 Clayton Ave, St Louis, MO 63110; telephone: (314) 977-0278; fax: (314) 977-0030; e-mail: suffman@cid.wustl.edu.

Ann Otol Rhinol Laryngol 110:2001

POLY(ADENOSINE DIPHOSPHATE–RIBOSE) SYNTHETASE INHIBITOR 3-AMINOBENZAMIDE ALLEVIATES COCHLEAR DYSFUNCTION INDUCED BY TRANSIENT ISCHEMIA

KEIJI TABUCHI, MD
ZENYA ITO, MD
SHIGEKI TSUJI, MD
AKIKO NAKAGAWA, MD
FUJIKO SERIZAWA, MD
AKIRA HARA, MD
JUN KUSAKARI, MD
TSUKUBA, JAPAN

The present study was undertaken to determine the possible deleterious role played by poly(adenosine diphosphate–ribose) synthetase (PARS) in cochlear ischemia-reperfusion injury. Transient ischemia of the cochlea was induced in albino guinea pigs for 15, 30, or 60 minutes by pressing the labyrinthine artery at the porus acusticus internus. The animals were given intravenous 3-aminobenzamide (a PARS inhibitor) or physiological saline solution 1 minute before the onset of reperfusion. The compound action potential thresholds were measured before the onset of ischemia and 4 hours after the onset of reperfusion. A statistically significant reduction in the postischemic compound action potential threshold shift was observed in the animals treated with 3-aminobenzamide after 15 or 30 minutes of ischemia, whereas no statistical difference was found after 60 minutes of ischemia. These results suggest that excessive activation of PARS exerts deleterious effects on the cochlear injury induced by transient ischemia.

KEY WORDS — 3-aminobenzamide, cochlea, compound action potential, ischemia, poly(adenosine diphosphate–ribose) synthetase.

INTRODUCTION

Poly(adenosine diphosphate [ADP]–ribose) synthetase (PARS), also referred to as poly(ADP-ribose) polymerase or transferase, is a nuclear enzyme, the expression of which is detected in almost all eukaryotic cells from plants to vertebrates. Breaks in DNA strands activate PARS to use nicotinamide adenine dinucleotide (NAD) to form a polymer of ADP-ribose on nuclear acceptor molecules such as histones, topoisomerase, and, predominantly, PARS itself. Although the physiological function of PARS is still poorly understood, its role under pathological conditions, particularly involving reactive oxygen species and nitric oxide (NO)–induced damage, has come to interest researchers. Reactive oxygen species,[1,2] NO,[3-5] and peroxynitrite[5,6] cause breaks in DNA strands and activate PARS. Excessive activation of PARS depletes NAD, which is a substrate of PARS.[1,4,6] Because NAD is essential to mitochondrial electron transport, its depletion interferes with adenosine triphosphate production and ultimately leads to cell death.[1,2,4,6]

Recently, it has been demonstrated that the formation of free radicals[7,8] and NO[9,10] contributes to ischemia-reperfusion injury of the cochlea. These findings strongly suggest that activation of PARS by free radicals and NO may also play an important role in this type of injury. To prove the involvement of PARS, we investigated the effect of 3-aminobenzamide (3AB), a prototypical inhibitor of PARS, on the cochlear dysfunction induced by transient ischemia.

MATERIAL AND METHODS

Animal Preparation. The studies were performed on 45 albino guinea pigs weighing 300 to 400 g with a normal Preyer pinna reflex. Anesthesia was induced by pentobarbital sodium (28 mg/kg by intraperitoneal injection; Nembutal, Abbott, North Chicago, Ill). The animals were tracheostomized, paralyzed with suxamethonium chloride (Relaxin, Kyorin, Tokyo, Japan), and artificially ventilated with room air. Body temperature was maintained at 37°C ± 1°C with a heating pad. Two catheters were used for each animal: one in the external jugular vein, for drug administration and fluid replacement, and the other in the right carotid artery, for monitoring blood pressure. An electrocardiogram and blood pressure were monitored throughout the experiment. All experimental procedures used in this study were approved by the Animal Experiment Committee at the University of Tsukuba under the Guidelines of the Declaration of Helsinki.

Measurement of CAP and Induction and Confirmation of Transient Ischemia. The methods used for

---

From the Department of Otolaryngology, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan. Supported by a grant from the Ministry of Education, Science and Culture (Grant-in-Aid for Scientific Research A(2) 09307039).

CORRESPONDENCE — Jun Kusakari, MD, Dept of Otolaryngology, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, 305-8575, Japan.

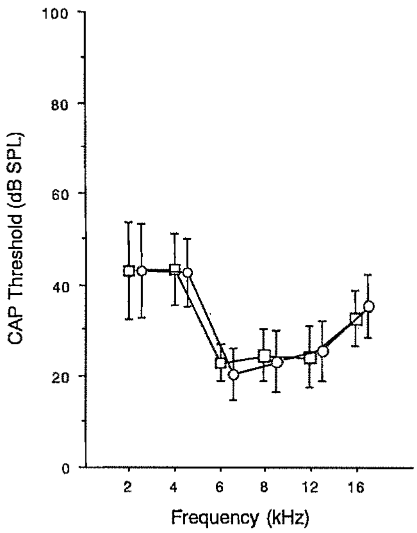


Fig 1. Compound action potential (CAP) thresholds before (squares) and 4 hours after (circles) administration of 3-aminobenzamide (3AB) at 10 mg/kg (n = 4; mean ± SD). Thresholds of CAP were not affected significantly by 3AB administration (2-way ANOVA, p > .05).

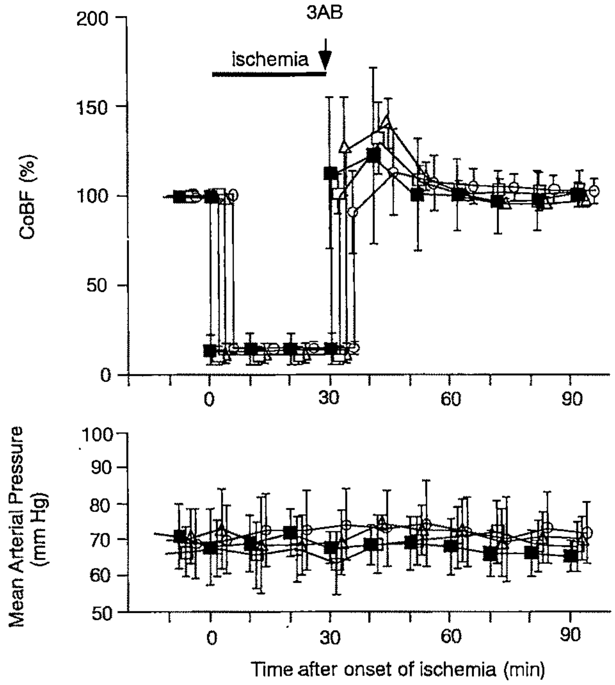


Fig 2. Graphs of cochlear blood flow (CoBF; percent) and mean arterial pressure (millimeters of mercury) before, during, and after onset of ischemia (mean ± SD) in 30-minute ischemia group. Closed squares indicate physiological saline solution (PSS) ischemia group, and open squares, triangles, and circles indicate 0.1-, 1-, and 10-mg/kg 3AB ischemia groups, respectively. Mean arterial pressure and CoBF were not affected by 3AB compared with control group (1-way ANOVA, p > .05).

measuring a compound action potential (CAP), inducing transient ischemia, and monitoring cochlear blood flow (CoBF) are described elsewhere.[8] In brief, CAPs were recorded at the bony edge of the round window by a silver–silver chloride electrode. The reference electrode was placed on the neck muscles. To evoke CAPs, bursts of pure tones from 2 to 16 kHz (rise and fall times, 1 ms; duration, 10 ms) were delivered in an open field system. Thirty-two, 64, or 128 responses were averaged, and the sound pressure level (SPL) necessary to evoke a CAP of 10 µV was defined as the threshold. When a stimulus of 110 dB SPL failed to evoke a potential of 10 µV, the threshold was considered to be 115 dB SPL to simplify calculations.

The basilar artery and its tributaries were exposed via the skull base approach. Cochlear ischemia was induced by pressing the labyrinthine artery at the porus acusticus internus with a metal stick.[11] A laser-Doppler flowmeter (BRL-100, Bio Research Center, Nagoya, Japan) was used to monitor the CoBF. Its flow probe was positioned at the lateral wall of the cochlear second turn.

Experimental Protocol. In the 3AB control group (n = 4), to examine whether administration of 3AB itself might affect the CAP thresholds, 10 mg/kg of 3AB was given intravenously and the CAP threshold was measured before and 4 hours after administration. In the physiological saline solution (PSS) ischemia group (n = 15), each animal was subjected to 15, 30, or 60 minutes of ischemia (5 in each subgroup) and intravenously received PSS at 2 mL/kg of body weight 1 minute before the onset of reperfusion. In the 3AB ischemia group (n = 26), each animal was intravenously given 3AB (dissolved in PSS at 2 mL/kg of body weight) 1 minute before the onset of reperfusion. The animals in this group were further divided into the following 3 subgroups according to the ischemic period: 1) the 15-minute ischemia group (n = 5), in which each animal was given 3AB at 10 mg/kg; 2) the 30-minute ischemia group (n = 16), in which each animal was given 3AB at 0.1 mg/kg (n = 5), 1 mg/kg (n = 5), or 10 mg/kg (n = 6); and 3) the 60-minute ischemia group (n = 5), in which each animal was given 3AB at 10 mg/kg.

The CAP thresholds were measured before the onset of ischemia and 4 hours after the onset of reperfusion. To evaluate the effect of 3AB on the cochlear dysfunction induced by transient ischemia, the CAP threshold shifts from the pre-ischemic values at 4 hours after the onset of reperfusion were compared between the PSS and the 3AB ischemia groups.

RESULTS

Effect of 3AB on CAP Threshold. Figure 1 shows the CAP threshold before and 4 hours after the administration of 3AB (mean ± SD, n = 4). Intravenous administration of 3AB (10 mg/kg) did not significant-

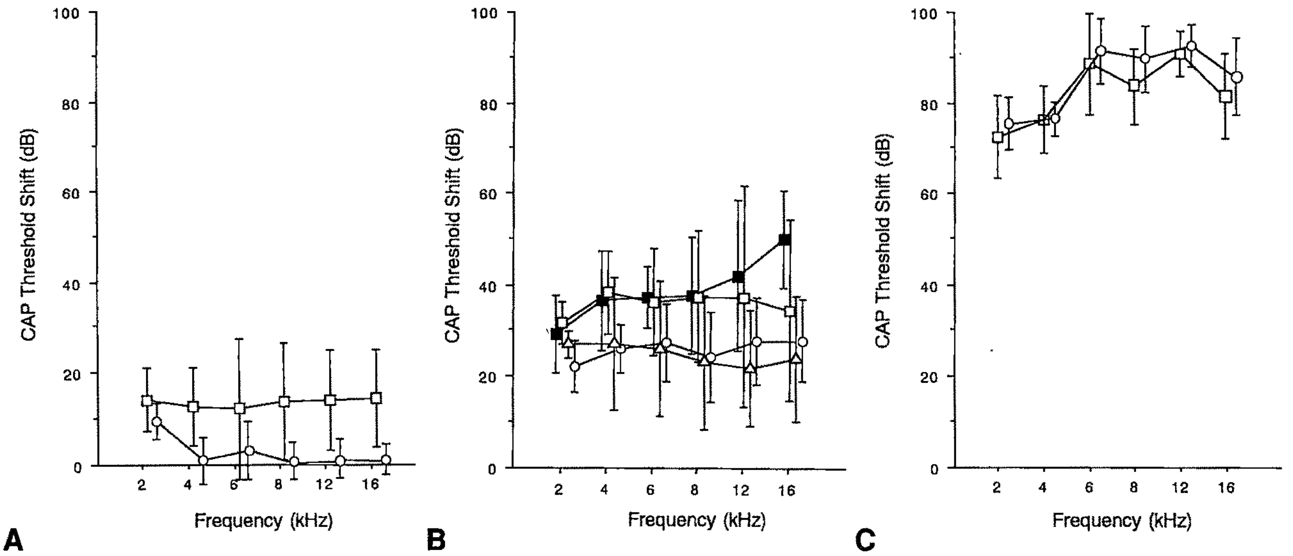


Fig 3. Threshold shifts in CAP from pre-ischemic values. Mean values ± SD 4 hours after restoration of cochlear blood circulation are plotted. A) 15-minute ischemia group. Squares and circles indicate PSS ischemia group and 10-mg/kg 3AB ischemia group, respectively. B) 30-minute ischemia group. Closed squares indicate PSS ischemia group, and circles, triangles, and open squares indicate 10-, 1-, and 0.1-mg/kg 3AB ischemia groups, respectively. C) 60-minute ischemia group. Squares and circles indicate PSS ischemia group and 10-mg/kg 3AB ischemia group, respectively.

ly affect the CAP thresholds (2-way analysis of variance [ANOVA]; F = 0.002, p > .05).

Cochlear Blood Flow During and After Transient Ischemia. Figure 2 illustrates changes in the CoBF and mean arterial pressure measured before, during, and after transient ischemia of 30 minutes' duration. The CoBF was plotted as a percentage relative to the value measured just before the onset of ischemia, and the postmortem value was set at 0%. As expected from the previous study,[8] the CoBF decreased rapidly when the labyrinthine artery was pressed and remained almost constant during ischemia. When the artery was unclamped, the CoBF promptly increased, with a transient overshoot, and then gradually regained a stable value that was close to the preischemic value. The mean arterial pressure and CoBF were not significantly affected by the administration of 3AB (1-way ANOVA; p > .05).

Effect of 3AB on CAP Threshold Shifts. Figure 3 shows the postischemic CAP threshold shifts measured 4 hours after the onset of reperfusion. After 15 minutes of ischemia, the CAP threshold shifts were significantly reduced in the 3AB ischemia group compared with the PSS ischemia group (2-way ANOVA; F = 25.7, p < .01; Fig 3A). Although 3AB at 0.1 mg/kg did not affect the postischemic CAP threshold shifts, 3AB at 1 or 10 mg/kg partially but significantly decreased the CAP threshold shifts in the 30-minute ischemia group (2-way ANOVA; F = 11.5, p < .01; Scheffé test; p < .01 in the 10-mg/kg subgroup, p < .01 in the 1-mg/kg subgroup, and p > .05 in the 0.1-mg/kg subgroup; Fig 3B). However, the CAP was almost completely abolished after 60 minutes of ischemia in both the 10-mg/kg 3AB group and the PSS ischemia group, and there was no statistically significant difference in the CAP threshold shifts between the two groups (2-way ANOVA; F = 2.5, p > .05; Fig 3C).

DISCUSSION

Recent studies suggest that reactive oxygen species (such as superoxide[7] and hydroxyl radical[8]) and NO[9,10] contribute to ischemia-reperfusion injury of the cochlea. Reactive oxygen species,[1,2] NO,[3-5] and peroxynitrite[5,6] have been proposed as powerful triggers of breaks in DNA strands and consequent excessive activation of PARS. Because PARS catalyzes the transfer of ADP-ribose moieties from NAD to nuclear acceptor molecules, excessive activation of PARS depletes the intracellular concentration of NAD and may lead to cell death.[1,4,6]

The major finding in the present study was 3AB's significant protection of the cochlear function against transient ischemia. This finding suggests that PARS plays a deleterious role in ischemia-reperfusion injury of the cochlea.

Although 3AB is the PARS inhibitor that has been most popularly used to inhibit PARS activity,[12] it may be argued whether the protective effect of 3AB demonstrated in the present study is due to its specific effect. The 3AB protects murine islet cells (wild-type) from cell death caused by oxygen-derived free radicals and NO, but failed in mutant cells whose PARS genes had been inactivated.[4] This finding indicates that the protective effect of 3AB against free radical–induced and NO-induced damage is a result of inhibition of PARS activity. In an in vivo experiment, 3AB significantly decreased the necrosis of the heart and the skeletal muscle caused by transient ischemia, whereas 3-aminobenzoic acid, an inactive analog of 3AB that does not inhibit PARS activity, did not significantly affect the necrosis volume after ischemia.[13] Furthermore, genetic disruption of PARS proved to be effective in alleviating ischemia-reperfusion injury in the brain.[14] Taking these findings into consideration, we believe that the protective effect of 3AB observed in the present study was a result of PARS inhibition.

Although the exact physiological functions of PARS remain unclear, it has been suggested that PARS might play a critical role in DNA repair,[15,16] because the activity of PARS is markedly increased after DNA damage.[17] Furthermore, an independent line of evidence had suggested a specific role of PARS in apoptotic cell death.[18] However, recent findings observed in PARS knock-out mice suggest that PARS is not essential for DNA repair and apoptotic cell death. Mutant embryonic fibroblasts were able to efficiently repair DNA,[19] and the sensitivity of hepatocytes and neurons to apoptosis was not altered by genetic disruption of PARS.[20] The PARS knock-out animals developed healthily, except for epidermal hyperplasia.[19] Provided that PARS is not necessarily essential to maintaining normal cell physiology, PARS inhibitors are possible candidates for treatment of ischemia-reperfusion injury.

In conclusion, 3AB, an inhibitor of PARS, exerted a protective effect on cochlear injury induced by transient ischemia, if the ischemic period was 30 minutes or shorter. Taking these findings into consideration, together with hitherto reported studies on PARS performed in other organs, we believe that excessive activation of PARS may play an important role in the generation of ischemia-reperfusion injury of the cochlea and that it may be a possible target for therapy.

REFERENCES

1. Schraufstatter IU, Hyslop PA, Hinshaw DB, Spragg RG, Sklar LA, Cochrane CG. Hydrogen peroxide–induced injury of cells and its prevention by inhibitors of poly(ADP-ribose) polymerase. Proc Natl Acad Sci U S A 1986;83:4908-12.

2. Hyslop PA, Hinshaw DB, Halsey WA, et al. Mechanisms of oxidant-mediated cell injury. J Biol Chem 1988;263:1665-75.

3. Zhang J, Dawson VL, Dawson TM, Snyder SH. Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. Nature 1994;263:687-9.

4. Heller B, Wang A-Q, Wagner EF, et al. Inactivation of the poly(ADP-ribose) polymerase gene affects oxygen radical and nitric oxide toxicity in islet cells. J Biol Chem 1995;270: 11176-80.

5. Cookson MR, Ince PG, Shaw PJ. Peroxynitrite and hydrogen peroxide induced cell death in the NSC34 neuroblastoma x spinal cord cell line: role of poly(ADP-ribose) polymerase. J Neurochem 1998;70:501-8.

6. Szabo C, Zingarelli B, O'Connor M, Salzman A. DNA strand breakage, activation of poly(ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity in macrophages and smooth muscle cells exposed to peroxynitrite. Proc Natl Acad Sci U S A 1996;93:1753-8.

7. Seidman MD, Quirk WS, Nuttall AL, Schweitzer VG. The protective effects of allopurinol and superoxide dismutase–polyethylene glycol on ischemic and reperfusion-induced cochlear damage. Otolaryngol Head Neck Surg 1991;105:457-63.

8. Tabuchi K, Ito Z, Wada T, Hara A, Kusakari J. The effect of mannitol upon cochlear dysfunction induced by transient local anoxia. Hear Res 1998;126:28-36.

9. Tabuchi K, Kusakari J, Ito Z, Wada T, Takahashi K, Hara A. Effect of nitric oxide synthase inhibitor on cochlear dysfunction induced by transient local anoxia. Acta Otolaryngol (Stockh) 1999;119:179-84.

10. Tabuchi K, Ito Z, Wada T, Takahashi K, Hara A, Kusakari J. Effect of 7-nitroindazole upon cochlear dysfunction induced by transient local anoxia. Ann Otol Rhinol Laryngol 2000;109: 715-9.

11. Kusakari J, Kambayashi J, Kobayashi T, et al. The effect of transient anoxia upon the cochlear potentials. Auris Nasus Larynx 1981;8:55-64.

12. Rankin PW, Jacobson EL, Benjamin RC, Moss J, Jacobson MK. Quantitative studies of inhibitors of ADP-ribosylation in vitro and in vivo. J Biol Chem 1989;264:4312-7.

13. Thiemermann C, Bowes J, Myint FP, Vane JR. Inhibition of the activity of poly(ADP-ribose) synthetase reduces ischemia-reperfusion injury in the heart and skeletal muscle. Proc Natl Acad Sci U S A 1997;94:679-83.

14. Eliasson MJL, Sampei K, Mandir AS, et al. Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nat Med 1997;3:1089-95.

15. Satoh MS, Lindahl T. Role of poly(ADP-ribose) formation in DNA repair. Nature 1992;356:356-8.

16. Satoh MS, Poirier GG, Lindahl T. NAD$^+$-dependent repair of damaged DNA by human cell extracts. J Biol Chem 1993; 268:5480-7.

17. Benjamin RC, Gill M. ADP-ribosylation in mammalian cell ghosts. J Biol Chem 1980;255:10493-501.

18. Nosseri C, Coppola S, Ghibelli L. Possible involvement of poly(ADP-ribosyl) polymerase in triggering stress-induced apoptosis. Exp Cell Res 1994;212:367-73.

19. Wang Z-Q, Auer B, Stingl L, et al. Mice lacking ADPRT and poly(ADP-ribosyl)ation develop normally but are susceptible to skin disease. Genes Dev 1995;9:509-20.

20. Leist M, Single B, Künstle G, Volbracht C, Hentze H, Nicotera P. Apoptosis in the absence of poly-(ADP-ribose) polymerase. Biochem Biophys Res Commun 1997;233:518-22.

Ann Otol Rhinol Laryngol 110:2001

CHANGES IN PERMEABILITY OF STRIAL VESSELS FOLLOWING VIBRATION GIVEN TO AUDITORY OSSICLE BY DRILL

MORIHIRO SEKI, MD
HIROE MIYASAKA, MD
HIDEO EDAMATSU, MD
KENSUKE WATANABE, MD

SAITAMA, JAPAN

We produced drill-induced damage of the auditory ossicles of guinea pigs to study changes over time in the permeability of the blood vessels of the stria vascularis to horseradish peroxidase (HRP). In group A, the stimulus was applied for 10 seconds after intravenous injection of HRP. In group B, it was applied for 30 seconds, and in group C, for 60 seconds. The cochlea was fixed with 2% glutaraldehyde perfused through the round window, and the guinea pigs were then decapitated. The stria vascularis of the basal and third turns was examined. The leakage of HRP from the blood vessels of the stria vascularis significantly increased in relation to the duration of the stimulus in both the basal and third turns. The damage to intermediate cells also tended to be in relation to the duration of the stimulus. Extravascular permeation of HRP took place through the tubules in the endothelial cytoplasm. The vibratory stimulation presumably opened channels that are not normally open.

KEY WORDS — drilling trauma, horseradish peroxidase, stria vascularis.

INTRODUCTION

It has been reported that sensorineural deafness occurs in 1.2% to 4.5% of patients after surgery of the middle ear.[1-3] Various factors appear to be involved: noise injury to the cochlea as a result of excessive drilling, instrumentation of the auditory ossicles, direct damage of the auditory ossicles with the drill, and perilymph or labyrinthine fistula.[3] Paparella[4] and Gjuric et al[5] have reported damage of the auditory ossicles, whereas there have been only a few in vivo studies of damage of the auditory ossicles with the passage of time. In any case, they considered that sensorineural hearing loss was caused by direct hair cell damage. No one has reported impairment of the stria vascularis caused by drilling.

In this study, we induced vibratory injury of the incus body by allowing a cutting bur of 1-mm diameter to touch the bone, in order to investigate changes in the blood vessels of the stria vascularis. To elucidate the process of damage to the blood vessels of the stria vascularis, the stimulus was applied for different times, and changes in the vascular permeability to horseradish peroxidase (HRP) and changes in the cells of the stria vascularis were examined.

METHODS

Fifteen guinea pigs with a normal Preyer's reflex and weighing 200 to 220 g were divided into 3 groups (groups A, B, and C). Each group consisted of 5 animals. The guinea pigs were anesthetized intraperitoneally with pentobarbital sodium (30 mg/kg), and the bilateral tympanic bullae were exposed and opened under a microscope. Horseradish peroxidase, 10 mg (type IV; Sigma Chemical Co, St Louis, Mo), was dissolved in 1 mL of distilled water, and the solution was injected into the femoral vein of the guinea pig. Then, a functioning drill was applied to the incus to induce vibration injury for 10 seconds in group A, for 30 seconds in group B, and for 60 seconds in group C. A cutting bur of 1-mm diameter was used at a rate of 10,000 rpm. After drilling, the cochlea was fixed with 2% glutaraldehyde solution perfused through the round window; the guinea pigs were painlessly decapitated 3 minutes later; and the portions of the stria vascularis corresponding to the basal and third turns were removed. According to a report by Xu et al,[6] if the cochlea of a physiologically normal guinea pig is fixed by perfusion before decapitation, practically no leakage of HRP from the stria vascularis occurs within 10 minutes, and for this reason, we completed the procedure from the intravenous injection of HRP until decapitation within 10 minutes. The collected specimens were further fixed in the same fixative and washed with 0.1-mol/L cacodylate buffer (pH 7.2; containing 8% sucrose) 3 times. Then, the specimens were incubated in diaminobenzidine solution (0.002% hydrogen peroxide and 8 mg of 3,3-diaminobenzidine dissolved in 10 mL of 0.05-mol/L Tris-acid buffer [pH 7.1]) at room temperature for 1 hour. The specimens were further washed with 0.1-mol/L cacodylate buffer 3 times and were post-fixed in 2% osmic acid for 40 minutes. After dehydration through a gradient of alcohol, the speci-

---

From the Department of Otorhinolaryngology, Koshigaya Hospital, Dokkyo University School of Medicine, Koshigaya, Japan.

CORRESPONDENCE — Morihiro Seki, MD, Dept of Otorhinolaryngology, Koshigaya Hospital, Dokkyo University School of Medicine, 2-1-50 Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan.

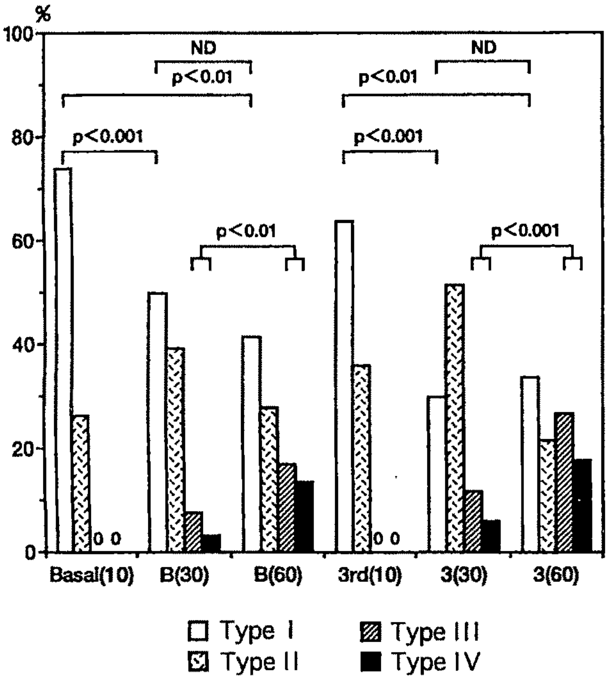


Fig 1. Rate of each type of capillary in basal (B) and third (3) turns of cochlea after different times of drilling. Numbers 10, 30, and 60 indicate seconds.

mens were embedded in Epon 812, sliced into ultrathin sections, double-stained with uranyl and lead, and observed under a transmission electron microscope (JEM-1200EX, JEOL). Thick sections were also prepared (1 to 2 µm thick) and stained with toluidine blue for observation under a light microscope.

RESULTS

Permeability of Blood Vessels of Stria Vascularis. The permeability of the blood vessels of the stria vascularis was classified into 4 types based on the status of HRP leakage.

1. Type I capillaries: no HRP leakage observed from the capillaries (blood vessels appear physiologically normal).
2. Type II capillaries: slight leakage of HRP.
3. Type III capillaries: leakage all around the blood vessels.
4. Type IV capillaries: leaked HRP also observed in the adjacent intercellular space.

To quantitatively study the types of permeability of the blood vessels of the stria vascularis, we took specimens at random from 3 sites of the basal and third turns in each animal, analyzed all blood vessels of the stria vascularis contained in the specimens, and calculated the percentages of each type of vessel (Fig 1).

A statistically significant difference was found in the percentages of type I capillaries between group A and group B (p < .001) and also between group A and group C (p < .01) in both the basal and third turns. In other words, the longer the duration of damage-inducing stimulation, the fewer the blood vessels retaining normal permeability. However, no statistically significant difference was noted in the percentage of type I blood vessels between group B and group C. We then compared group B and group C with regard to the percentage of blood vessels presenting distinct extravascular HRP leakage (types III and IV). In group C, the percentage of such blood vessels was significantly higher in both the basal (p < .01) and the third turns (p < .001), indicating that HRP leakage was greatest in group C.

Virtually no difference was found in the percentage of such blood vessels between the basal and the third turns. However, in group B, the percentage of type I capillaries was significantly higher (p < .001) in the basal turn than in the third turn.

Changes in Intermediate Cells of Stria Vascularis. Changes were also observed in the intermediate cells after vibration by drilling. The intermediate cells of the stria vascularis were classified into 2 types based on their morphology: 1) type A: normal, and 2) type B: atrophic and degenerated.

As shown in Fig 2, in group A, all (100%) interme-

Fig 2. Changes of intermediate cells after drilling.

	Basal	3rd
Group A (10 seconds)	Type A (100%)	Type A (100%)
Group B (30 s)	A (100%)	A (80%) | B (20%)
Group C (60 s)	A (40%) | B (60%)	A (20%) | B (80%)

Type A : Normal intermediate cells
Type B : Atrophic or degenerated intermediate cells

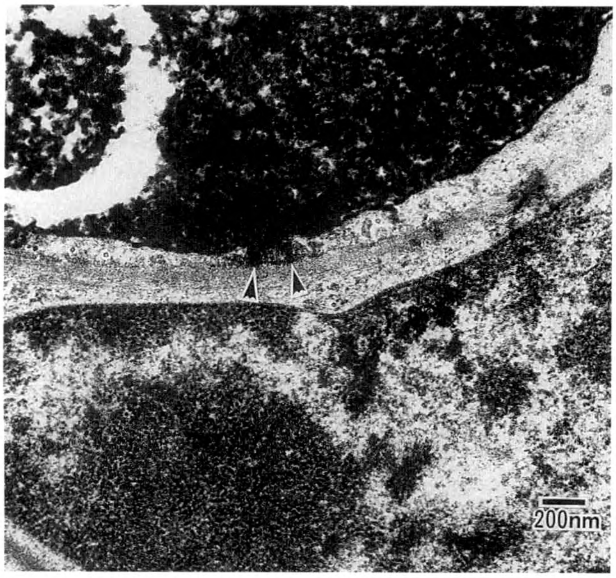


Fig 3. Stria vascularis of basal turn after 10-second vibratory stimulation. This capillary is type I. No extravascular horseradish peroxidase (HRP) leakage is observed, but HRP is found in tubules inside endothelial cells (arrowheads).

diate cells were of type A in both the basal and third turns. In group B, all intermediate cells were of type A in the basal turn; in the third turn 80% of the cells were of type A, and 20% were of type B. In group C, 40% were of type A, and 60% were of type B in the basal turn; in the third turn, only 20% of the cells were of type A, and 80% were of type B. These findings indicated that the extent of damage of the intermediate cells also depended on the duration of drill-induced vibration, and that cell damage tended to be more severe in the upper turn.

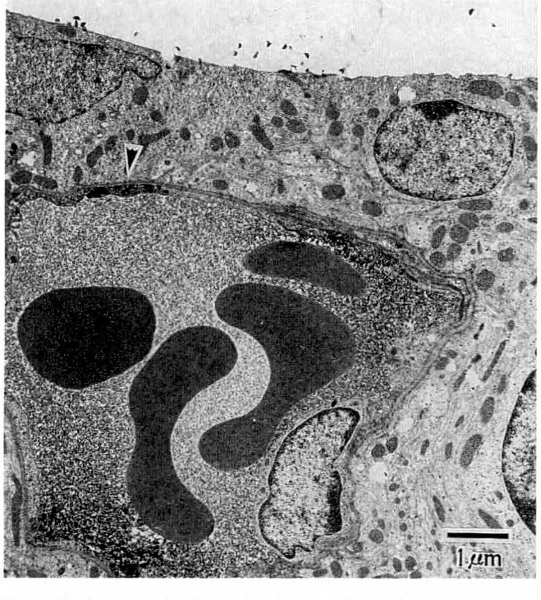


Fig 4. Stria vascularis of third turn after 10-second vibratory stimulation. This capillary is type II, in which slight extravascular HRP leakage is observed. HRP is observed scattered inside endothelial cells (arrowhead).

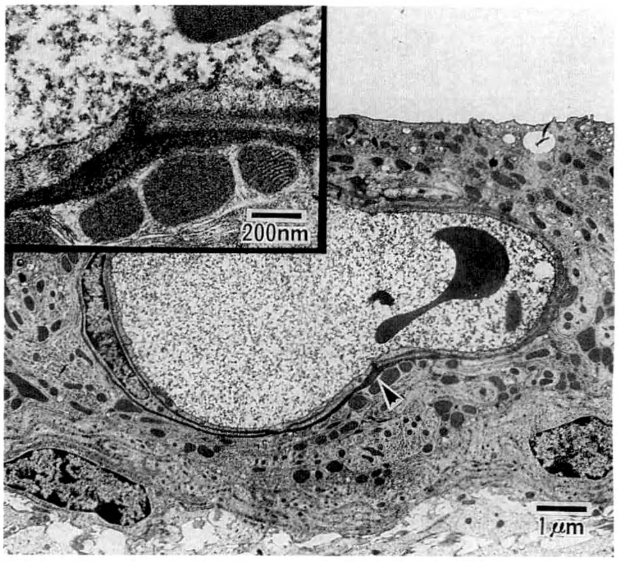


Fig 5. Stria vascularis of third turn after 30-second vibratory stimulation. This capillary is type III, in which HRP leakage is observed all around vessels. Inset) High magnification of area indicated with arrowhead. Large amount of HRP has leaked into extravascular space through tubules of endothelial cells.

Channels for Extravascular HRP Leakage. The channels by which extravascular leakage of HRP occurred were studied under an electron microscope.

Figure 3 is an electron micrograph of the basal turn taken after 10 seconds of vibratory stimulation. The blood vessels are of type I, and no extravascular HRP leakage is observed, but HRP is found in the microtubules inside the endothelial cells (arrowheads). Slight diffusion of HRP into the endothelial cytoplasm is also seen. In some capillaries of type II, HRP is seen scattered inside the endothelial cells (Fig 4, arrowhead). In the blood vessels of type III, leaked HRP is observed all around the vessels (Fig 5).

In type IV capillaries, a large amount of HRP is observed around the capillaries and in the intercellular space of the stria vascularis (Fig 6). A large amount of HRP has leaked into the extravascular space, apparently through the tubules (Fig 5, inset; Fig 7, arrowhead) of the endothelial cells. Moreover, HRP has extensively diffused inside the endothelial cells, and the endothelial cytoplasm is filled with a substance of high electron density (Fig 7, arrow). Some intermediate cells are degenerated (Fig 6, arrow) around the type III and IV capillaries.

DISCUSSION

There are various mechanisms for the onset of acute sensorineural deafness following surgery of the middle ear. Plenty of experimental data and clinical evidence are available on the etiologic mechanisms,

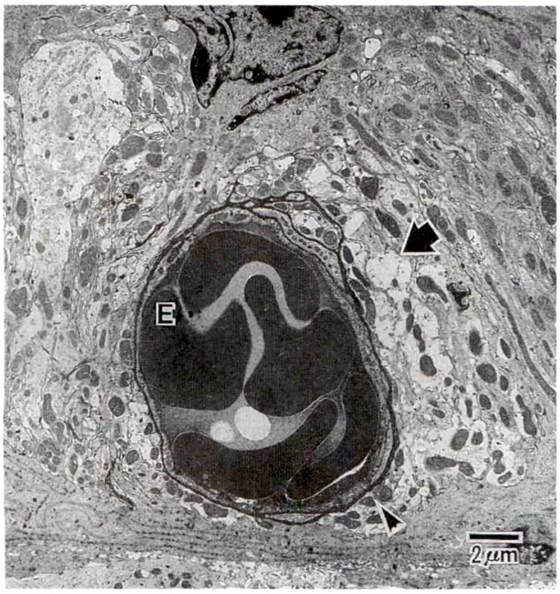


Fig 6. Stria vascularis of basal turn after 60-second vibratory stimulation. This capillary is type IV, in which large amount of HRP is observed in intercellular space of stria vascularis (arrowhead). Intermediate cells are destroyed (arrow). E — erythrocyte.

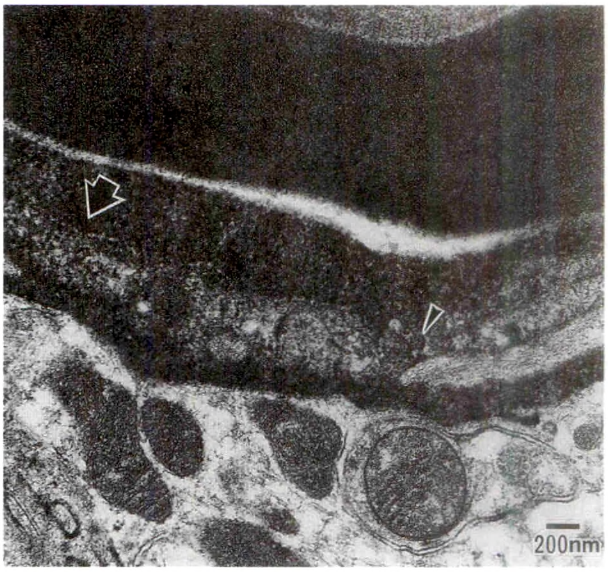


Fig 7. High magnification of area indicated by arrowhead in Fig 6. Large amount of HRP has leaked into extravascular space through site that appears to be tubules (arrowhead) of endothelial cells. Moreover, HRP has extensively diffused inside endothelial cells, and endothelial cytoplasm is filled with substance of high electron density (arrow).

whereas there are only a few reports on inadvertent damage to the auditory ossicles that occurs during drilling inside the epitympanic or middle ear cavity.[4,5] Paparella[4] showed that a 4-mm cutting bur applied to the malleus head or the body of the incus of a cat produces injury to the organ of Corti, located in the lower basal turn. Auditory behavioral testing revealed an average hearing loss of about 30 dB in 3 animals, and a 55-dB loss in the fourth, and that no damage of the cochlea occurred when the cutting bur was 0.5 mm in diameter. Gjuric et al[5] induced damage to the body of the guinea pig incus by stimulation for 10 seconds with a diamond bur of 1.4 mm in diameter, and measured electrocochleographically the effect of the damage on hearing. They reported that within several seconds of drilling, the hearing threshold value varied; that irreversible deafness developed after 10-second stimulation; and that no spontaneous recovery of this deafness occurred during a 5-week observation period.

In our study, on stimulation of the auditory ossicles with a cutting bur of 1 mm in diameter for 10 seconds, distinct HRP leakage from the blood vessels of the stria vascularis (type III or type IV) was not observed in either the first or third turn. According to the data without drill stimulation reported by Xu et al,[6] however, 80.9% of strial capillaries showed no HRP leakage (type I) in the first turn, and 90.3% in the third turn, which were higher than the rates of type I capillaries after 10-second drilling in this study (74% in the basal turn, and 64% in the third turn). It was therefore evident that even 10-second stimulation caused slight HRP leakage. Statistically distinct HRP leakage occurred after 30-second or 60-second stimulation. A statistically significant difference was also noted between the 30-second and the 60-second stimulations, indicating that the degree of HRP leakage depended on the duration of stimulation. Moreover, there was a high percentage of destroyed intermediate cells after 60-second stimulation, which suggested that irreversible deafness might potentially have occurred in this group.

Because our present study was focused on inner ear impairment caused by induced vibration of the auditory ossicles, auditory injury caused by noise was not evaluated. Schuknecht and Tonndorf[7] assumed that the intensity of noise generated by the rotating drill bur was safe. However, in reports on noise injury of the cochlea that occurs during drilling on the temporal bone, it has been shown that transient variations in hearing threshold value usually occur.[8-13] Kylen and Arlinger[12] reported drill-induced noise levels with various kinds, sizes, and rotation rates of the drill bur in otologic surgery. According to their report, the noise level varied chiefly with the size of the bur. If a high noise level, such as one exceeding 100 dB, lasts for 1 to 2 hours, it will greatly affect auditory acuity. In our study, the duration of stimulation was only 1 minute; thus, it had hardly any deleterious effects on the cochlea. However, it appears necessary to study the effect of noise on the blood vessels of the stria vascularis in the future.

The HRP used in this study was introduced for the first time in 1966 as a research tracer for vascular permeability at ultrastructural levels.[14] Duvall et al[15] and Winther[16] later used HRP to observe the permeability of blood vessels of the stria vascularis, and reported that the stria vascularis was very permeable to HRP. However, Watanabe[17] demonstrated that massive leakage of HRP from the blood vessels of the stria vascularis was an artifact due to blood sludge resulting from the shock of decapitation. Watanabe showed that if experimental animals are decapitated after the cochlea has been perfused for fixation so that the blood vessels do not contract on decapitation, no blood sludge occurs in the blood vessels of the stria vascularis and no extravascular permeation of HRP is observed. Xu et al[6] observed very little extravascular leakage of HRP 10 minutes after intravenous HRP injection. Therefore, we decapitated the guinea pigs after fixation of the cochlea by perfusion within 10 minutes of intravenous injection of HRP. The HRP leaks into the extravascular space from the blood vessels of the stria vascularis, as reported by Xu et al,[6] by micropinocytosis under physiologically normal conditions, whereas under nonphysiological conditions, HRP leaks into the extravascular space not only through many pinocytotic vesicles after shock,[17] but also through openings of the endothelial intercellular space of the blood vessels of the stria vascularis after administration of endotoxin into the middle ear cavity.[18] The leakage of HRP took place through an entirely different channel in this study. After 10-second stimulation, little extravascular leakage of HRP was observed, but HRP was found in the tubules inside the endothelial cells. No HRP is found in such tubules of endothelial cells of normal blood vessels, and presumably, some channels opened because of the drill-induced vibration, allowing HRP to flow in. More interestingly, HRP was found to diffuse from the tubules into the endothelial cytoplasm. As the vibratory stimulation was prolonged to 30 and 60 seconds, HRP diffused extensively into the endothelial cytoplasm, and a large amount of HRP also leaked to the extravascular space through the tubules. There is no report on such a channel for extravascular leakage of HRP, and it may be that the vibration caused sudden, drastic damage to the endothelial cells, resulting in the opening of some channel for the leakage. According to the leakage pattern of HRP observed under the electron microscope, the frequency of patency of the tubules appears to be related to the duration of vibratory stimulation. It is of particular interest to know what other kinds of damage can open such channels, and it appears necessary to comparatively study various stimulations as potential causes of this phenomenon.

REFERENCES

1. Palva T, Karja J, Palva A. High-tone sensorineural losses following chronic ear surgery. Arch Otolaryngol 1978;98:176-8.

2. Smyth GDL. Sensorineural hearing loss in chronic ear surgery. Ann Otol Rhinol Laryngol 1977;86:3-8.

3. Tos M, Lau T, Plate S. Sensorineural hearing loss following chronic ear surgery. Ann Otol Rhinol Laryngol 1984;93:403-9.

4. Paparella M. Acoustic trauma from the bone cutting bur. Laryngoscope 1962;72:116-26.

5. Gjuric M, Schneider W, Wolf SR, Wigand ME. Experimental sensorineural hearing loss following drill-induced ossicular chain injury. Acta Otolaryngol (Stockh) 1997;117:497-500.

6. Xu RD, Watanabe K, Komatsuzaki A. Permeability for horseradish peroxidase in strial capillaries in each turn of cochlea. ORL J Otorhinolaryngol Relat Spec 1994;56:183-9.

7. Schuknecht HF, Tonndorf J. Acoustic trauma of the cochlea from ear surgery. Laryngoscope 1960;70:479-505.

8. Urquhart AC, McIntosch WA, Bodenstein NP. Drill-generated sensorineural hearing loss following mastoid surgery. Laryngoscope 1992;102:689-92.

9. Domenech J, Carulla M, Traserra J. Sensorineural high-frequency hearing loss after drill-generated acoustic trauma in tympanoplasty. Arch Otorhinolaryngol 1989;246:280-2.

10. Hegewald M, Heitman R, Wiederhold ML, Cooper JC, Gates GA. High-frequency electrostimulation hearing after mastoidectomy. Otolaryngol Head Neck Surg 1989;100:49-56.

11. Hüttenbrink KB. Die Cochlea schädigung durch mittelohr-operationen. Laryngorhinootologie 1991;70:66-71.

12. Kylen P, Arlinger S. Drill-generated noise levels in ear surgery. Acta Otolaryngol (Stockh) 1976;82:402-29.

13. Suits GW, Brummett RE, Nunley J. Effect of otologic drill noise on ABR thresholds in a guinea pig model. Otolaryngol Head Neck Surg 1993;109:660-7.

14. Graham RC, Karnovsky MJ. The early stage of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney. J Histochem Cytochem 1966;14:291-302.

15. Duvall AJ, Quick CA, Sutherland CR. Horseradish peroxidase in the lateral cochlea wall. Arch Otolaryngol 1971;93:304-16.

16. Winther FO. The permeability of the guinea pig cochlea capillaries to horseradish peroxidase. Z Zellforsch 1971;114:193-202.

17. Watanabe K. Change in the capillary permeability of the stria vascularis by different methods of death and fixation. Ann Otol Rhinol Laryngol 1986;95:427-31.

18. Watanabe K, Tanaka Y. Horseradish peroxidase permeation from the capillaries of the stria vascularis after inoculation of endotoxin into the middle ear. Ann Otol Rhinol Laryngol 1997;106:394-8.

Ann Otol Rhinol Laryngol 110:2001

VESTIBULAR FINDINGS IN PROFESSIONAL DIVERS

ZOHARA SHARONI, MA
HAIFA, ISRAEL

AVI SHUPAK, MD
HAIFA, ISRAEL

ORNA SPITZER, MA
HAIFA, ISRAEL

ZOHAR NACHUM, MD
HAIFA, ISRAEL

NATAN GADOTH, MD
KFAR-SABA, ISRAEL

The purpose of the present study was to investigate possible inner ear changes related to professional diving, by the documentation of auditory and vestibular function in 13 asymptomatic professional divers and 12 nondiver controls. A higher average pure tone hearing threshold, although of no clinical significance, was found in the study group (8.53 ± 4.85 versus 6.67 ± 3.54 dB hearing level, $p = .04$). In the vestibular evaluation, the smooth harmonic acceleration test phase leads for 0.01, 0.02, and 0.04 Hz were significantly lower in the divers (0.01 Hz, 38.46° ± 7.15° versus 45.83° ± 9.02°, $p = .02$; 0.02 Hz, 21.08° ± 5.19° versus 25.17° ± 5.78°, $p = .05$; 0.04 Hz, 12.38° ± 3.69° versus 14.25° ± 3.14°, $p = .05$). We suggest that the lower smooth harmonic acceleration phase values found in the professional divers, reflecting longer vestibulo-ocular reflex primary time constants and enhancement of the velocity storage mechanism, are the result of a habituation process that augments the low-frequency response of the canal-ocular system.

KEY WORDS — professional diving, sinusoidal harmonic acceleration, velocity storage, vestibular adaptation, vestibular function tests, vestibulo-ocular reflex.

INTRODUCTION

Professional divers are generally engaged in diving to great depths for extended periods of time, using specialized gas mixtures rather than simply compressed air. The underwater environment, which is characterized by rapidly changing ambient pressures and raised inert gas tensions, presents an increased risk to the inner ear structures.[1,2] Previous studies have reported hearing deterioration in divers. When hearing test results were compared between 160 professional divers and 160 nondivers matched for age and sex, more divers were found to suffer from hearing loss, especially at the higher frequencies (4 to 8 kHz).[3] In another study, 60% of the divers were found to suffer from sensorineural, high-tone hearing loss.[4] Two large-scale surveys[5,6] reported that hearing loss was the most frequent otolaryngological problem in divers. Divers also suffered from earlier and more severe hearing deterioration as compared with nondivers. These findings may be related to noise exposure, middle or inner ear barotrauma, or inner ear decompression sickness.

The most frequent injuries that result from scuba and surface-supplied diving are barotrauma and decompression sickness.[2,7] Inner ear barotrauma is related to difficulties in middle ear pressure equalization. It occurs mostly during the descent phase of the dive, in which the raised ambient pressure leads to a relative underpressure in the tympanic cavity (according to Boyle's law, which states that at a constant temperature, the volume of a given mass of gas is inversely proportional to the pressure). When middle ear clearing fails and a pressure differential greater than 90 mm Hg builds up between the nasopharynx and the middle ear, the eustachian tube is usually blocked and locked. Under these conditions, the raised intracranial pressure might be transmitted to the inner ear through a patent or semipatent cochlear aqueduct. These pressure forces can cause the rupture of Reissner's membrane or the basilar membrane and/or a labyrinthine window fistula, with consequent impairment of inner ear function: hearing deterioration, dizziness, and tinnitus.[8-11]

Inner ear decompression sickness occurs mostly after deep diving or after switching inert gases during the dive.[11,12] However, it can also result from recreational scuba diving.[13] Inner ear decompression sickness is related to the formation and growth of inert gas bubbles within microvessels and otic fluids, which takes place whenever pressure drops rapidly during the ascent from a dive to a level lower than that required to keep gas soluble.[7] Extravascular bubble formation in the otic fluids may cause additional damage due to tearing of blood vessels and

---

From the Israel Naval Medical Institute, Israel Defense Forces Medical Corps (Sharoni, Shupak, Spitzer, Nachum), and the Department of Otolaryngology–Head and Neck Surgery, Carmel Medical Center (Shupak), Haifa, and the Department of Neurology, Sapir Medical Center, Kfar-Saba (Gadoth), Israel.

CORRESPONDENCE — Zohara Sharoni, MA, Israel Naval Medical Institute, PO Box 8040, 31 080 Haifa, Israel.

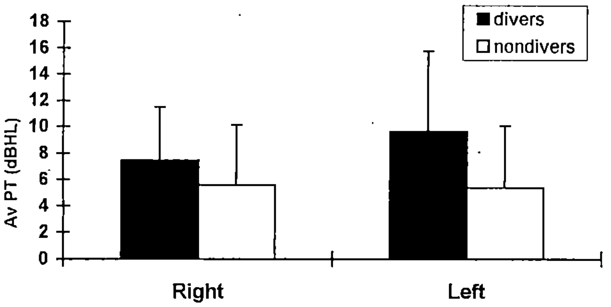


Fig 1. Average pure tone (Av PT) threshold (mean ± SD) for 3, 4, and 6 kHz.

inner ear membranes.[14]

The long-term effects of diving on the vestibular system have not yet been investigated. The purpose of the present study was to investigate possible inner ear changes related to professional diving by the documentation of auditory and vestibular function in professional divers and nondiver controls.

SUBJECTS AND METHODS

The study population included all 13 professional divers available to us. They were all healthy, were free of any vestibular or audiological symptoms, and had no history of any clinically significant diving event related to barotrauma or decompression sickness. All 13 subjects were men, and their average age (±SD) was 31.76 ± 7.9 years. The control group included 12 male nondivers of similar age, with no history of hearing or vestibular impairment. The audiological and vestibular examinations were conducted at least 48 hours after the most recent dive to avoid any acute changes that might be attributed to diving.

All subjects in both the study and control groups underwent the following tests.

The audiological test battery included pure tone air and bone conduction, speech reception threshold, and discrimination audiometry with a GSI16 audiometer (Grason-Stadler, Inc, Milford, NH) and tympanometry with a GS33 middle ear analyzer (Grason-Stadler, Inc).

The vestibular test battery included electronystagmography (ENG) and the smooth harmonic acceleration (SHA) rotatory chair test. Computerized ENG was carried out with the Neurokinetics ENG system (Neurokinetics, Inc, Pittsburgh, Pa). Horizontal and vertical eye movements were recorded during the gaze, positional, positioning, and alternate bithermal water caloric tests. Canal paresis and directional preponderance were calculated.[15] The SHA test was performed with the Neurokinetics computerized rotatory chair system. As previously described,[16] the SHA test evaluates the simultaneous function of both horizontal semicircular canals in response to a series of angular accelerations ranging in frequency from 0.01 to 0.16 Hz. The following calculated vestibulo-ocular reflex (VOR) parameters reflect the response to these accelerations: VOR gain is the ratio of the eye's maximal slow-component velocity to the maximal head rotation velocity; VOR phase describes the timing of eye movements relative to the head velocity input, and is expressed as the number of degrees by which the peak eye velocity leads or lags behind the peak head velocity. Asymmetry is the percentage difference in maximal eye slow-component velocity in response to clockwise and counterclockwise rotation. Spectral purity is the percentage of the slow-component output power that is at the stimulus frequency. The remainder of the response is harmonic distortion or noise. Only if the spectral purity is greater than 75% can the gain, phase, and asymmetry be considered accurate descriptions of the VOR.[16]

Statistical analysis was carried out with SPSS software (SPSS, Inc, Chicago, Ill) on an IBM personal computer. The Wilcoxon-Mann-Whitney rank sum test was used to compare the average pure tone and speech audiometry hearing levels, the average slow-component eye velocity in the ENG caloric test, and the average VOR parameters calculated in the SHA test. Pearson's χ^2 test was used to compare the occurrence of ENG gaze, positional, and positioning nystagmus. In the interpretation of the data, an α level of .05 was used as the critical level of significance.

RESULTS

The average (±SD) pure tone hearing loss was higher in the divers: 8.53 ± 4.85 dB hearing level (HL) versus 6.67 ± 3.54 dB HL in the nondivers (p = .05, Wilcoxon rank test). This difference was even more evident at the higher frequencies (3 to 6 kHz). The average pure tone hearing loss at 3, 4, and 6 kHz was higher in the divers: right ear, 7.44 ± 4.06 dB HL versus 5.56 ± 4.56 dB HL; left ear, 9.62 ± 6.17 dB HL versus 5.37 ± 4.70 dB HL (Fig 1). These results were of statistical significance for the left ear only (p = .04, Wilcoxon-Mann-Whitney rank sum test).

In the speech reception test, there was no significant difference between the two groups.

All tympanometry test results were normal, indicating that all study and control group participants had normal middle ear compliance and ventilation.

In the vestibular tests, none of the subjects had a clinically abnormal evaluation according to the criteria adopted by the vestibular laboratory of Israel

DIFFERENCES BETWEEN GROUPS IN ELECTRONYSTAGMOGRAPHY

Test	Study Group	Control Group	p
Gaze, center	None	2 (16.7%)	NS
Gaze, right	2 (15.4%)	None	NS
Gaze, left	1 (7.7%)	1 (8.3%)	NS
Gaze, down	1 (7.7%)	None	NS
Dix-Hallpike maneuver	7 (53.8%)	5 (41.7%)	NS
Positional nystagmus	5 (38.5%)	4 (33.3%)	NS
Directional preponderance	3.66 ± 22.41	4.71 ± 10.56	NS
Canal paresis lateralization	–4.4 ± 17.19	2.83 ± 15.09	NS

For gaze, Dix-Hallpike maneuver, and positional nystagmus, Table refers to number of subjects in whom such responses were found. Statistical analysis was by χ^2 test. For caloric test results (directional preponderance and lateralization), Table refers to average percentage. Statistical analysis was by Wilcoxon-Mann-Whitney rank sum test.

NS — not significant.

Naval Medical Institute.[15,17]

In the ENG recordings, we found minor and inconsistent differences between the groups for the various types of nystagmus. These recordings did not differ significantly between the groups, nor did the canal paresis or directional preponderance parameters of the caloric test (see Table).

The SHA phase leads for the lower frequencies examined were significantly lower in the divers (Fig 2A). At 0.01 Hz, the average phase lead in the divers was 38.46° ± 7.15°, and in the nondivers, 45.83° ± 9.02° ($p = .02$, Mann-Whitney-Wilcoxon). At 0.02 Hz, the average phase lead in the divers was 21.08° ± 5.19°, and in the nondivers, 25.17° ± 5.78° ($p = .05$, Mann-Whitney-Wilcoxon). At 0.04 Hz, the average phase lead in the divers was 12.38° ± 3.69°, and in the nondivers, 14.25° ± 3.14° ($p = .05$, Mann-Whitney-Wilcoxon). At 0.08 and 0.16 Hz, the differences between the groups did not reach statistical significance.

The gain values in all of the subtests were higher in the divers than in the nondivers. This difference was statistically significant only for 0.01 Hz (0.34 ± 0.09 in the divers and 0.27 ± 0.08 in the nondivers; $p = .03$, Mann-Whitney-Wilcoxon). At the higher frequencies, the differences between the groups did not reach statistical significance (Fig 2B).

No differences in SHA asymmetry or spectral purity parameters were found between the groups.

DISCUSSION

The purpose of the present study was to document possible vestibular changes related to professional diving, by comparing a group of professional divers with no history of inner ear injury and a group of nondivers.

The average pure tone hearing threshold was significantly higher in the divers than in the nondivers. These results are in accord with the findings of previous studies[3-7,18] that reported hearing deterioration in professional divers, especially at the higher frequencies. Although the divers' hearing threshold in our study was higher than that of the nondivers, all individual hearing test results were within normal limits. This finding may therefore be of no clinical significance.

In the ENG test, we did not find significant differences between the groups, nor did we find evidence of clinically relevant disease in any of the study participants. This observation concurs with those of previous studies in which no disease could be documented in the ENG of professional divers engaged in deep or saturation diving.[18] The most significant finding in this study was the difference between the groups in the VOR phase values of the SHA test. The SHA phase leads for the lower frequencies examined (0.01, 0.02, and 0.04 Hz) were significantly lower in the divers. Lower phase values reflect prolongation of the VOR primary time constant and enhancement of the velocity storage mechanism, in-

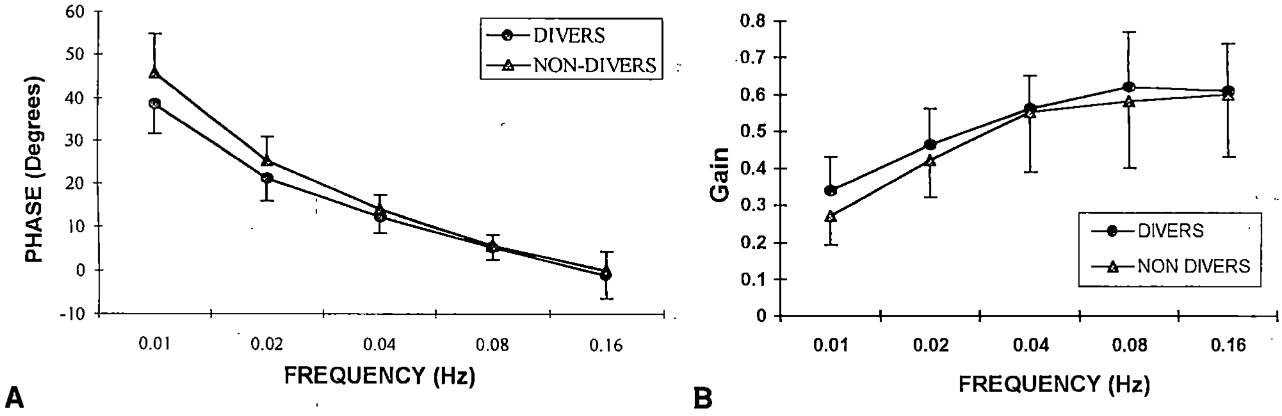


Fig 2. Smooth harmonic acceleration A) phase lead and B) gain (mean ± SD).

creasing the low-frequency response of the canal-ocular system. The velocity storage mechanism is the neural activity that matches head and eye movement velocity beyond the limited response dictated by the cupula-endolymph mechanics of the semicircular canals. The nerve firing time constant of the semicircular canals enables effective synchronization between head and eye movements. This time constant cannot on its own, however, explain eye movements in response to head oscillations at frequencies below 0.02 Hz. If the vestibular response were dependent only on the semicircular canal nerve firing rate, it would have faded after a few seconds, because at a steady-state velocity the cupula returns to its natural state after 5 to 7 seconds. In this situation, the firing rate of the primary vestibular neurons, which increases according to the angle of deviation of the cupula, no longer reflects head movement.[19] When the head movement frequency is low, it is the velocity storage mechanism that enables the eyes to track head movements. A possible, though purely speculative, explanation for the low phase values we found in the professional divers is the need to accommodate the VOR to the new head motion characteristics encountered when the head is submersed in water. In water, natural head movements will be damped by the density of the surrounding medium, which is higher than that of air. In these conditions, existing VOR parameters, which normally match head and eye movements for the frequency range 0.5 to 8 Hz, will be rendered inappropriate for the new head movements, which are in a lower frequency range.[20-22] We suggest that in order to make a longer VOR response possible, adaptation of the velocity storage mechanism takes place, so that the VOR primary time constant can be further enhanced for the low-frequency response. This change may be reflected in lower phase values in the SHA low-frequency tests. The VOR gain values were found to be higher in the divers, reaching statistical significance for the subtest at 0.01 Hz. This inverse ratio between gain and phase values has been described previously.[23,24]

The VOR may be modulated in response to changes in the optokinetic or vestibular stimulus. This plasticity has previously been demonstrated for exposure to new motion patterns, such as rough sea conditions, and unusual visual stimuli, such as the wearing of reversing prisms.[20-22,25,26] Since the coordination of head and eye movements by the VOR on land does not extend below the frequency of 0.5 Hz, the response to the low frequency range of 0.01 to 0.16 Hz examined by the SHA test might reflect the changes that occur so that the body can cope with the new conditions created by the underwater environment. Recent studies have demonstrated that this adaptation of the VOR is context-specific and will be activated only on exposure to the specific frequencies that bring it about.[27,28]

The only parameter for vestibular modification examined in our study was that of the horizontal VOR characteristics produced by the semicircular canals. Velocity storage changes might also be introduced by the optokinetic oculomotor system in response to underwater changes in visual stimuli.[29] Such changes can be estimated by measuring the optokinetic afternystagmus time constant.[30] Unfortunately, difficulties attributed to the initial low slow-component velocity of the human optokinetic afternystagmus, and high test-retest variability, make such measurements impractical.[30,31] Adaptation of the VOR is probably the most important mechanism in the balance compensation process for natural terrestrial head movement frequencies of 0.5 to 8 Hz.[27] However, where low-frequency motion patterns are concerned, interactions with the otolith organs and the visual and proprioceptive systems will also contribute to the required modifications in balance control. These might include the cervico-ocular reflex, the pursuit system, the central preprogramming of eye motion, and the somatosensory and other sensory systems.[28,32]

In conclusion, we found no evidence of vestibular disease in any of the professional divers examined in this study. Lower VOR phase values, reflecting longer VOR primary time constants, were found in the divers than in nondivers. This modulation of the VOR might be necessary for the production of correctly matched compensatory eye-to-head movements during submersion. Our understanding of the strategy by which the vestibular system copes with changing balance requirements underwater might be broadened by an investigation of possible contributions by the otolith organs and the visual and proprioceptive systems to compensation. Ideally, such changes can be monitored by a close follow-up of the diver's balance function throughout his or her training and with evolving diving experience.

REFERENCES

1. Melamed Y, Shupak A, Bitterman H. Medical problems associated with underwater diving. N Engl J Med 1992;326:30-5.

2. Madsen J, Hink J, Hyldegaard O. Diving physiology and pathophysiology. Clin Physiol 1994;14:597-626.

3. Zannini D, Odaglia G, Sperati G. Auditory changes in professional divers. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the Fifth Symposium on Underwater Physiology. Bethesda, Md: FASEB, 1976:675-84.

4. Edmonds C. Hearing loss with frequent diving (deaf divers). Undersea Biomed Res 1985;12:315-9.

5. Molvaer OI, Lehmann EH. Hearing acuity in professional

divers. Undersea Biomed Res 1985;12:333-49.

6. Molvaer OI, Albrektsen G. Hearing deterioration in professional divers: an epidemiologic study. Undersea Biomed Res 1990;17:231-46.

7. Shupak A, Doweck I, Greenberg E, et al. Diving-related inner ear injuries. Laryngoscope 1991;101:173-9.

8. Goodhill V, Harris I, Brockman SJ, Hantz O. Sudden deafness and labyrinthine window ruptures. Audio-vestibular observations. Ann Otol Rhinol Laryngol 1973;82:2-12.

9. Caruso VG, Winkelmann PE, Correia MJ, Miltenberger GE, Love JT. Otologic and otoneurologic injuries in divers: clinical studies on nine commercial and two sport divers. Laryngoscope 1977;87:508-21.

10. Parell GJ, Becker GD. Inner ear barotrauma in scuba divers. A long-term follow-up after continued diving. Arch Otolaryngol Head Neck Surg 1993;119:455-7.

11. Edmonds C. Vertigo in diving. In: Guedry FE Jr, ed. AGARD Conference proceedings No. 128. The use of nystagmography in aviation medicine. Neuilly sur Seine, France: North Atlantic Treaty Organization, Advisory Group for Aerospace Research and Development, 1973;A24:1-10.

12. Farmer JC Jr. Otological and paranasal sinus problems in diving. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving. 4th ed. London, England: WB Saunders, 1993:267-300.

13. Reissman P, Shupak A, Nachum Z, Melamed Y. Inner ear decompression sickness following a shallow scuba dive. Aviat Space Environ Med 1990;61:563-6.

14. McCormick JG, Holland WB, Brauer RW, Holleman IL Jr. Sudden hearing loss due to diving and its prevention with heparin. Otolaryngol Clin North Am 1975;8:417-30.

15. Barber HO, Stockwell CW. Manual of electronystagmography. 2nd ed. St Louis, Mo: CV Mosby, 1980.

16. Jacobson GP, Newman CW. Rotational testing. Semin Hear 1991;12:199-215.

17. National Research Council, Commission on Behavioral Sciences and Education, Committee on Hearing, Bioacoustics and Biomechanics (CHABA), Working Group on Evaluation of Tests for Vestibular Function. Evaluation of tests for vestibular function. Aviat Space Environ Med 1992;63(suppl):A1-A34.

18. Cross M, Mayo I. Audiometric and vestibular function studies in a population of North Sea divers. In: Smith G, ed. Proceedings of the Sixth International Congress on Hyperbaric Medicine. Aberdeen, Scotland: Aberdeen University Press, 1977:431-3.

19. Sharpe JA, Johnston JL. The vestibulo-ocular reflex: clinical, anatomic, and physiologic correlates. In: Sharpe JA, Barber HO, eds. The vestibulo-ocular reflex and vertigo. New York, NY: Raven Press, 1993:15-39.

20. Melvill Jones G, Davies P. Adaptation of cat vestibulo-ocular reflex to 200 days of optically reversed vision. Brain Res 1976;103:551-4.

21. Gonshor A, Melvill Jones G. Extreme vestibulo-ocular adaptation induced by prolonged optical reversal of vision. J Physiol (Lond) 1976;256:381-414.

22. Powell KD, Peterson BW, Baker JF. Phase-shifted direction adaptation of the vestibulo-ocular reflex in cat. J Vestib Res 1996;6:277-93.

23. Shupak A, Kerem D, Gordon C, Spitzer O, Mendelowitz N, Melamed Y. Vestibulo-ocular reflex as a parameter of seasickness susceptibility. Ann Otol Rhinol Laryngol 1990;99:131-6.

24. Gordon CR, Spitzer O, Doweck I, Shupak A, Gadoth N. The vestibulo-ocular reflex and seasickness susceptibility. J Vestib Res 1996;6:229-33.

25. Young LR, Oman CM. Model for vestibular adaptation to horizontal rotation. Aerosp Med 1969;40:1076-80.

26. Malcolm R, Melvill Jones G. A quantitative study of vestibular adaptation in humans. Acta Otolaryngol (Stockh) 1970; 70:126-35.

27. Herdman SJ. Role of vestibular adaptation in vestibular rehabilitation. Otolaryngol Head Neck Surg 1998;119:49-54.

28. Kramer P, Shelhamer M, Zee DS. Short-term vestibulo-ocular adaptation: influence of context. Otolaryngol Head Neck Surg 1998;119:60-4.

29. Butler FK Jr. Diving and hyperbaric ophthalmology. Surv Ophthalmol 1995;39:347-66.

30. Tijssen MAJ, Straathof CSM, Hain TC, Zee DS. Optokinetic afternystagmus in humans: normal values of amplitude, time constant, and asymmetry. Ann Otol Rhinol Laryngol 1989; 98:741-6.

31. Hain TC, Herdman SJ, Holliday M, Mattox D, Zee DS, Byskosh AT. Localizing value of optokinetic afternystagmus. Ann Otol Rhinol Laryngol 1994;103:806-11.

32. Brandt T, Strupp M, Arbusow V, Dieringer N. Plasticity of the vestibular system: central compensation and sensory substitution for vestibular deficits. Adv Neurol 1997;73:297-309.

Ann Otol Rhinol Laryngol 110:2001

IMMORTALIZATION OF RAT MIDDLE EAR EPITHELIAL CELLS BY ADENO 12–SV40 HYBRID VIRUS

SHIGEHIRO UEYAMA, MD
OITA, JAPAN

SUNJI JIN, MD
YANJI CITY, CHINA

TOMOYO UEYAMA, MD
OITA, JAPAN

JOHNG S. RHIM, MD
BETHESDA, MARYLAND

DAVID J. LIM, MD
LOS ANGELES, CALIFORNIA

Rat middle ear epithelial cells were infected with the adeno 12–SV40 hybrid virus. The cell line thus obtained displays features of primary cultured epithelial cells in both light microscopic and ultrastructural examinations. The immortalized cells have been in continuous proliferation for 40 passages and more than 17 months. Immunohistochemical analysis of the immortalized cells was positive for the SV40 T antigen and the tumor suppressor protein p53. The cells also stained positive for cytokeratin, an epithelial cell marker, and negative for vimentin, a fibroblast marker. These results, together with karyotype analysis, indicate that this cell line originated from rat middle ear epithelial cells and retains the characteristics of epithelial cells. This cell line will be useful for studying the normal cellular biology of middle ear epithelial cells, as well as the cellular and molecular mechanisms involved in the bacteria–middle ear epithelial cell interaction.

KEY WORDS — adeno 12–SV40 hybrid virus, cell culture, cell line, immortalization, rat middle ear epithelial cells.

INTRODUCTION

It has been proposed that bacterial colonization of the upper respiratory tract, and subsequent bacteria–epithelial cell interaction in the middle ear, play an important role in the pathogenesis of otitis media. Attempts to further define the molecular mechanisms of otitis media have been hampered by the need for large quantities of middle ear epithelial cells. In order to obtain a pure population of middle ear epithelial cells, primary cultures of the middle ear epithelium of rats,[1] guinea pigs,[2] gerbils,[3,4] and chinchillas[5-7] have been developed in recent years. The analysis of these cultured middle ear epithelial cells has significantly contributed to a better understanding of the biology of middle ear epithelial cells. There is a need for the development of middle ear epithelial cell lines from laboratory animals and humans. In the past, there has been only 1 report of successful immortalization of middle ear epithelial cells, in the gerbil.[8] The aim of this study was to establish a rat middle ear epithelial cell line in order to investigate the normal biology of the middle ear epithelial cells, as well as cellular and molecular mechanisms involved in bacteria-host interaction. The rat middle ear epithelial cell was chosen because of the availability of large numbers of markers in rats. We report the establishment of a successful cell line of rat middle ear epithelial cells from primary culture by viral infection with the adeno 12–SV40 hybrid virus. This cell line has been characterized by morphological, immunohistochemical, and biochemical methods.

MATERIALS AND METHODS

Animals. A total of 20 healthy male rats (Wistar-Kyoto), with body weights ranging from 150 to 200 g, 4 to 5 weeks of age, without middle ear infection were obtained from Taconic (Germantown, New York).

Harvest of Middle Ear Epithelium. Under general anesthesia, with intramuscular ketamine hydrochloride (Fort Dodge Laboratories, Inc, Fort Collins, Colorado) at a dose of 40 mg/kg, the rats were painlessly sacrificed by cervical dislocation and decapitated. Middle ear mucosae were aseptically harvested from the tympanic bullae. These tissues were chopped into fine pieces and immersed in saline solution with a mixture of penicillin G sodium (100 U/mL), strep-

---

From the Laboratory of Cellular Biology, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Rockville (S. Ueyama, Jin, T. Ueyama, Lim), and the Laboratory of Biochemical Physiology, National Cancer Institute, National Institutes of Health, Frederick (Rhim), Maryland, and the Gonda Department of Cell and Molecular Biology, House Ear Institute, Los Angeles, California (Lim). Dr Ueyama is currently at the Funai ENT Clinic, Oita, Japan. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the National Institute on Deafness and Other Communication Disorders at the National Institutes of Health.

CORRESPONDENCE — David J. Lim, MD, House Ear Institute, 2100 W Third St, Fifth Floor, Los Angeles, CA 90057.

tomycin sulfate (100 µg/mL), and amphotericin B (250 µg/mL) for 1 hour at 4°C before being used as explants.

Culture Medium. The culture medium was essentially the same as that used by Van Blitterswijk et al[1] and Nakamura et al,[5] with minor modifications. Our culture medium consisted of a mixture of 3 parts Dulbecco's modified Eagle's medium (Life Technologies, Gaithersburg, Maryland) and 1 part nutrient mixture F12 (Ham's F12, Life Technologies) supplemented with 5% fetal bovine serum (Life Technologies), hydrocortisone (0.4 µg/mL; Sigma, St Louis, Missouri), isoproterenol (10^{-6} mol/L; Sigma), penicillin G sodium (100 U/mL; Life Technologies), streptomycin sulfate (100 µg/mL; Life Technologies), and amphotericin B (250 µg/mL; Life Technologies). If small numbers of fibroblasts were found in the outgrowth in some dishes, they were physically removed with a scalpel under the microscope. In addition, a period of serum-free incubation was carried out when fibroblast contamination was observed. Typically, after 4 to 5 weeks, no further fibroblast contamination was seen, and serum was then added back to the culture media. If fibroblast contamination was again observed, such dishes were discarded.

Primary Explants. Several explants were placed on a 35-mm tissue culture dish coated with collagen I (BioCoat, Bedford, Massachusetts) in a small volume of culture medium, initially without epidermal growth factor (EGF). The dishes were incubated at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air. Up to 2 mL of new medium was added to the culture the next day. When outgrowth was seen, EGF (10 µg/mL; Sigma) was added to the culture medium. The culture medium was changed twice a week.[1,5]

Primary Cell Culture. We used collagen I–coated petri dishes to promote the attachment of explants to the culture plates. From the 1st to the 6th passages, the cells were cultured with serum-supplemented culture medium. When the cultured cells were found to have fibroblast contamination, fibroblasts were eliminated with a scalpel and/or by use of serum-free medium for several passages. After the disappearance of fibroblasts, serum-supplemented culture medium was again used to maintain the primary culture of the pure population of epithelial cells.

Immortalization. Middle ear epithelial cells were cultured for 14 to 21 days in 35-mm culture dishes. After reaching 80% confluence, cultured epithelial cells were infected with a 1:10 dilution of adeno 12–SV40 hybrid virus and incubated for 3 days.[9,10] Both infected and control cells were cultured to allow comparison of growth rates. On the 56th day after the inoculation of adeno 12–SV40 hybrid virus, subculture of the infected cells was begun. The cells were subcultured at a 1:2 ratio once a week. In order to assess whether the infected epithelial cell cultures were still shedding infectious virus, supernatants from the cultures were added to a fresh culture of Vero cells. The Vero cell cultures were examined for the appearance of characteristic cytopathic changes after 3 weeks.[11,12] At the 17th passage, cloning of all infected cells was carried out to select the well-growing culture cells. After selection of such cells as a potential cell line, characterization experiments were undertaken.

Morphological Study. For light microscopic analysis, immortalized middle ear epithelial cells were cultured on collagen I–coated coverslips (BioCoat), placed in petri dishes, examined with an inverted phase contrast microscope (Axiovert 135, Zeiss, Thornwood, New York), and photographed.

Electron Microscopy. For scanning electron microscopy (SEM), the cells were cultured on glass coverslips (BioCoat) and then rinsed with phosphate-buffered saline solution (PBS, pH 7.4) and fixed with 4% glutaraldehyde in 0.1-mol/L sodium cacodylate buffer for 2 hours at room temperature. After washing in cacodylate buffer, the cells were immersed in a thiocarbohydrazide (TCH)–saturated solution for 30 minutes, followed by incubation in 1% osmium tetroxide for 3 hours. This TCH and osmium treatment was repeated. The specimens were then dehydrated in graded ethanol, critical point dried with carbon dioxide, sputter coated with gold, and subsequently observed by SEM (S-2460N, Hitachi, Tokyo, Japan).

For transmission electron microscopy (TEM), cells were grown on plastic coverslips (Nunc, Naperville, Illinois). Specimens were fixed with 4% sodium cacodylate-buffered glutaraldehyde for 2 hours at room temperature. After washing in cacodylate buffer, they were postfixed in 1% osmium tetroxide for 3 hours and dehydrated in graded alcohol. The specimens were immersed in propylene oxide and embedded in Epon, then cured in a 60°C oven for 12 hours. Ultrathin sections were then made, double-stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope (100 CX, JEOL, Sundberg, Sweden).

Immunohistochemistry. Indirect immunofluorescence was used to detect SV40 T antigen, p53 cytokeratin, and vimentin. Cells were cultured on collagen I–coated coverslips (BioCoat) for immunohistochemical staining. They were fixed with pre-chilled methanol (–20°C) for 20 minutes and permeabilized with acetone (4°C) for 2 minutes and dried, then rinsed with PBS. The coverslips with cultured cells were incubated with 10% normal rat serum (Calbiochem, San Diego, California) in PBS for 1 hour to block nonspecific antigens. After washing with PBS, mouse monoclonal antibodies against p53 (Oncogene Science, Uniondale, New York), and mouse monoclonal antibodies against SV40 large T antigen (Oncogene Science) were used to confirm the insertion of virus gene to the host genome. Anti-ZO-1 immunostaining using monoclonal antibody (Chemicon International, Inc, Temecula, California) was performed by the procedure described above. The cultured cells were also stained with mouse monoclonal anti-cytokeratin antibody C-11 (Sigma) and monoclonal anti-vimentin antibody VIM-13.2 (Sigma) to identify the cell line as epithelial cells. The specificity of these monoclonal antibodies was previously established by the vendors. As a negative control, the slides were incubated for 1 hour with 5% normal mouse serum (Calbiochem) instead of mouse monoclonal antibodies. Excess antibody was rinsed off with PBS, after which the coverslips were covered with fluorescein isothiocyanate (FITC)–labeled goat anti-mouse IgG (Oncogene Science) and incubated for 1 hour at 37°C. After washing of excess antibodies, the coverslips were mounted on slide glass in glycerin gel. The slides were examined with a light microscope equipped with incident-light fluorescence (Zeiss, Axiovert 135).

Cytokeratin Staining Pattern. The cytokeratin staining patterns of primary and immortalized middle ear epithelial cells were analyzed. The following 12 types of monoclonal cytokeratin antibodies (Sigma), which were made from 15 clones, were used: 1) monoclonal antibody 6B10, which is specific for cytokeratin polypeptide 4 and reacts with noncornifying squamous epithelium[13]; 2) monoclonal antibody LDS-68, which reacts with cytokeratin polypeptide 7 and specifically with most simple epithelia[14]; 3) monoclonal antibody CKB1, which is specific for cytokeratin polypeptide 14 and labels the basal layer of certain squamous epithelia (eg, exocervical, laryngeal epithelium)[15]; 4) monoclonal antibody CK-E3, which is specific for cytokeratin polypeptide 17 and labels a minor basal cell population in all variants of "complex" epithelia[16]; 5) monoclonal antibody CY-90, which is specific for cytokeratin polypeptide 18 and reacts with simple epithelium[17]; 6) monoclonal antibody K4.62, which is specific for cytokeratin polypeptide 19 and reacts with noncornified epithelium and respiratory epithelium[18,19]; 7) monoclonal antibody K8.60, which is specific for cytokeratin polypeptides 10 and 11 and labels suprabasal layers of epidermis, as well as other squamous epithelia[18]; 8) monoclonal antibody K8.12, which is specific for cytokeratin polypeptides 13, 15, and 16 and labels nonkeratinizing squamous epithelium, the basal layer of pseudostratified epithelium, and transitional epithelium[20]; 9) monoclonal antibody PCK-26, which is specific for cytokeratin polypeptides 1, 5, 6, and 8 and reacts with simple, cornifying and noncornifying squamous epithelia and pseudostratified epithelium[21]; 10) broad-spectrum monoclonal antibody C-11, which reacts with cytokeratin polypeptides 4, 5, 6, 8, 10, 13, and 18[22-24]; 11) broad-spectrum monoclonal antibody K8.13, which reacts with cytokeratin polypeptides 1, 5, 6, 7, 8, 10, 11, and 18[18]; and 12) broad-spectrum monoclonal antibody, a mixture of C-11, PCK-26, CY-90, KS-1A3, M20, and A53-B/A2, which reacts with a broad range of polypeptides, including Nos. 1, 4, 5, 6, 8, 10, 13, 18, and 19, and most epithelial cells.[17,21-24] The staining procedure was the same as that described above.

BrdU Staining (Cell Proliferation Analysis). The cell proliferation status of the cultured immortalized and primary cells was monitored with the anti-5-bromo-2'-deoxyuridine (BrdU) kit RPN 20 (Amersham Co, Arlington Heights, Illinois), as BrdU incorporates into the DNA of dividing cells.[25] We added BrdU into the culture media of primary and immortalized cells on coverslips and incubated them for 45 minutes at 37°C. The cells were fixed in acid-alcohol for 30 minutes. After washing with PBS, the cells were incubated with anti-BrdU monoclonal antibodies plus nuclease for 1 hour. The cells were then rinsed again with PBS, treated with peroxidase-conjugated anti-mouse IgG2a antibodies to detect the BrdU-labeled cells, and, after treatment with substrated peroxidase solution, counterstained with eosin.

Western Blot Analysis. Subconfluent cultures of the appropriate cell clones were washed with PBS (pH 7.2) at 4°C and suspended in lysate buffer, consisting of 0.1-mol/L sodium chloride, 0.001-mol/L ethylenediaminetetraacetic acid, 0.01-mol/L Tris chloride (pH 7.6), aprotinin (1 mg/mL; Sigma), and 2-mmol/L phenylmethylsulfonyl fluoride (Sigma) at 4°C. After mixing with lysate buffer, a loading buffer containing 100-mmol/L Tris.Cl (pH 6.8), 200-mmol/L dithiothreitol, 4% sodium dodecyl sulfate (SDS), 0.2% bromophenol blue, and 20% glycerol was added and mixed gently. The sample was boiled for 10 minutes, and the protein concentration was then adjusted to the appropriate level (20 µg/10 µL). The sample was loaded onto a 6% polyacrylamide SDS gel. After electrophoresis, proteins on the gel were electroeluted onto a nitrocellulose membrane that was then reacted with mouse monoclonal antibodies against p53 or against SV40 large T antigen for 60 minutes at room temperature. The membrane was washed in Tris-buffered saline solution and Tween 20 (TBST) and was incubated with alkaline phosphate–conju-

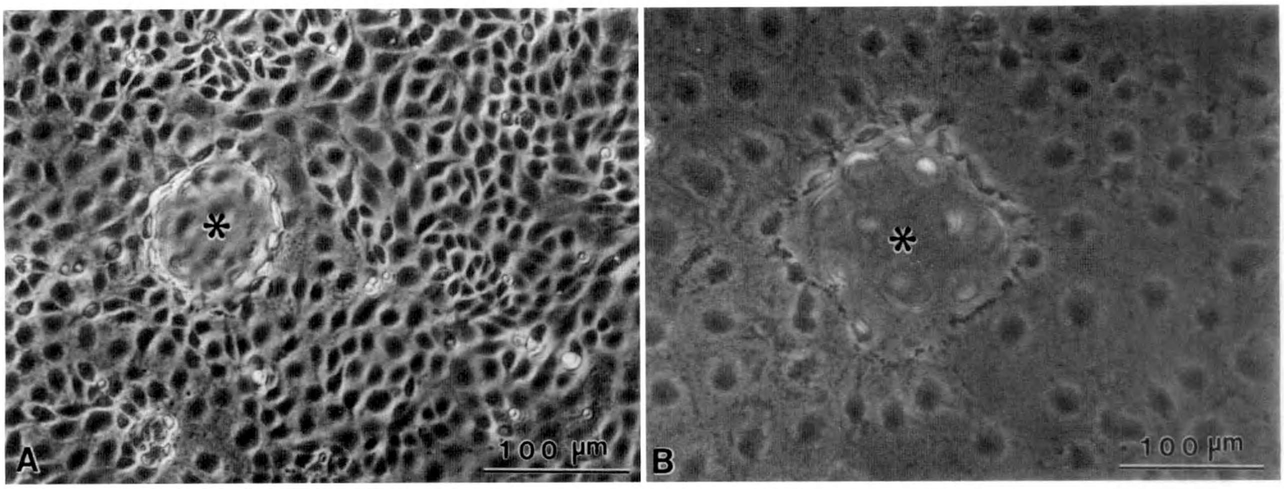


Fig 1. Phase-contrast light microscopic photomicrographs (original ×200). A) Immortalized rat middle ear epithelial cells. B) Primary culture of rat middle ear epithelial cells. Both primary and immortalized cells show cobblestone appearance and "dome" formation (asterisks). Immortalized cells were considerably smaller than primary cultured cells.

gated anti-mouse IgG antibody for 60 minutes at room temperature. After washing in TBST, the membrane was immersed in a substrate solution.

Chromosomal Analysis. Karyotypic analysis was performed by Dr Joseph Kaplan, Jr, Children's Hospital of Michigan, Detroit, Michigan.

Growth Rate of Immortalized Cells. The number of generations of exponential growth was calculated[10]: $N = (No.)(2^x)$, where N is the final number of cells, No. is the initial number of cells, and x is the number of generations. Then, the doubling time was calculated by the equation $DT = T/G$, where DT is the doubling time, T is the total time elapsed, and G is the number of generations.

Colony Formation in Soft Agar. A cell suspension (1×10^4 cells per mL) was made in 5 mL of 0.35% Noble agar (Difco, Detroit, Michigan) with medium. This gel was then transferred onto a 60-mm dish containing a 0.6% agar base. At 21 days, viable colonies were checked.

RESULTS

Immortalization. A successful outgrowth of the rat middle ear epithelial cells was obtained. Primary middle ear cells that survived 55 days from the day of inoculation with adeno 12–SV40 hybrid virus were then subcultured on the 56th day. The parent control cells (primary culture) underwent senescence by 87 days within 3 passages; however, the immortalized cells have continued to proliferate for more than 40 passages and have been maintained for more than 14 months. From passage 4 to passage 8, we checked to see whether the infected cells had ceased shedding the infectious virus at every subculture. No infectious virus was detected from the supernatants. At the 17th passage, the selection of a high-growth cell was accomplished by cloning. The well-grown cultures were selected and maintained and designated as RMEE-1. Vero cell cultures inoculated with a supernatant from the RMEE-1 cell showed no evidence of cytopathic effects.

Light Microscopy. The primary cultures showed several features of epithelial cells, including monolayer "dome" formation and cobblestone appearance. The dome formation or bulging of the epithelial cell sheet is due to the collection of fluid underneath, and the cobblestone appearance is the result of the development of tight junctions.[5] From passage 13 to passage 15, a few "dome formations" began to appear after confluency (Fig 1). Dome formations suggest that transepithelial fluid transport has occurred.[8] Ciliated cells were seen at the initial stage of primary culture and gradually disappeared. Ciliated cells and goblet cells were not expressed in later passages. The growth of immortalized cells was robust, especially after 6 passages. The cultured cells grew stably after the 6th passage. The average cell length of the immortalized cells (n = 50) was 17.54 ± 2.48 µm (mean ± SD), as opposed to 59.57 ± 15.61 µm for the primary cells (n = 50).

Electron Microscopy. The SEM findings showed that the immortalized cells and primary cultured cells revealed features of epithelial cells, such as a cell monolayer expressing polygonal arrangement and tight junctions (Fig 2). The TEM findings of immortalized middle ear cells grown on a plastic coverslip showed a single layer of cells forming tight junctions. The cells had mature junctional complexes, well-developed rough endoplasmic reticulum (rER), numerous ribosomes, Golgi apparatus, microvilli, mitochondria, and tonofilaments, and frequent lysosomal granules (Fig 3A,B). The TEM findings of pri-

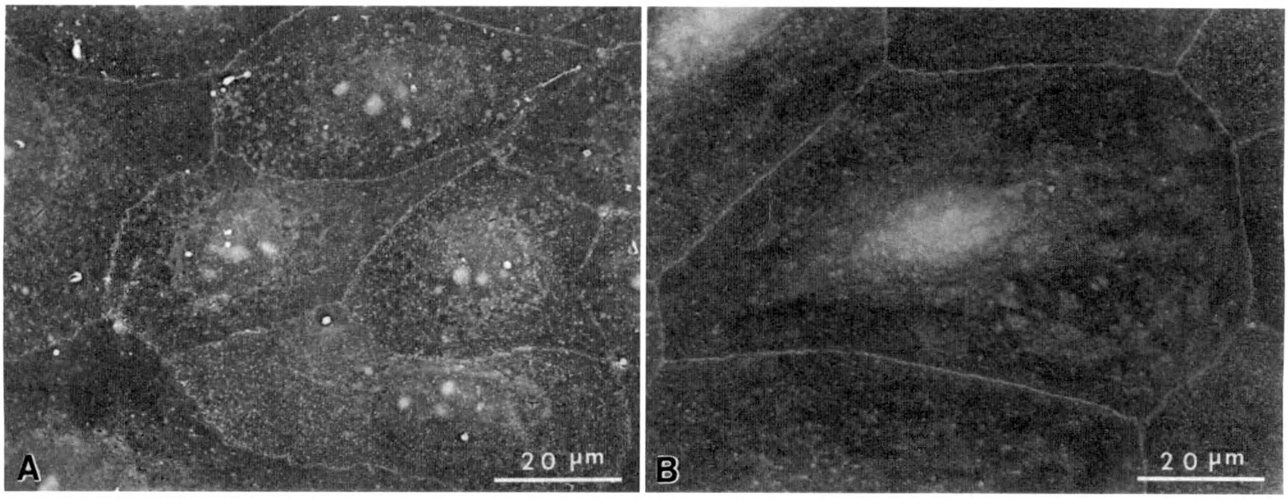


Fig 2. Scanning electron micrographs (original ×1,000). A) Immortalized rat middle ear epithelial cells. B) Primary culture of rat middle ear epithelial cells. Both primary and immortalized cells maintain roughly hexagonal or heptagonal cell shapes.

mary cultured cells revealed a monolayer of cells that contained large ovoid nuclei, mitochondria, well-developed rER, numerous ribosomes, Golgi apparatus, microvilli, abundant tonofilaments and desmosomes, and lysosomal granules (Fig 3C,D). The findings from the immortalized cells were comparable to those of the primary cultured cells.

Immunohistochemistry. The results of the cytokeratin staining pattern in the primary cultured cells and

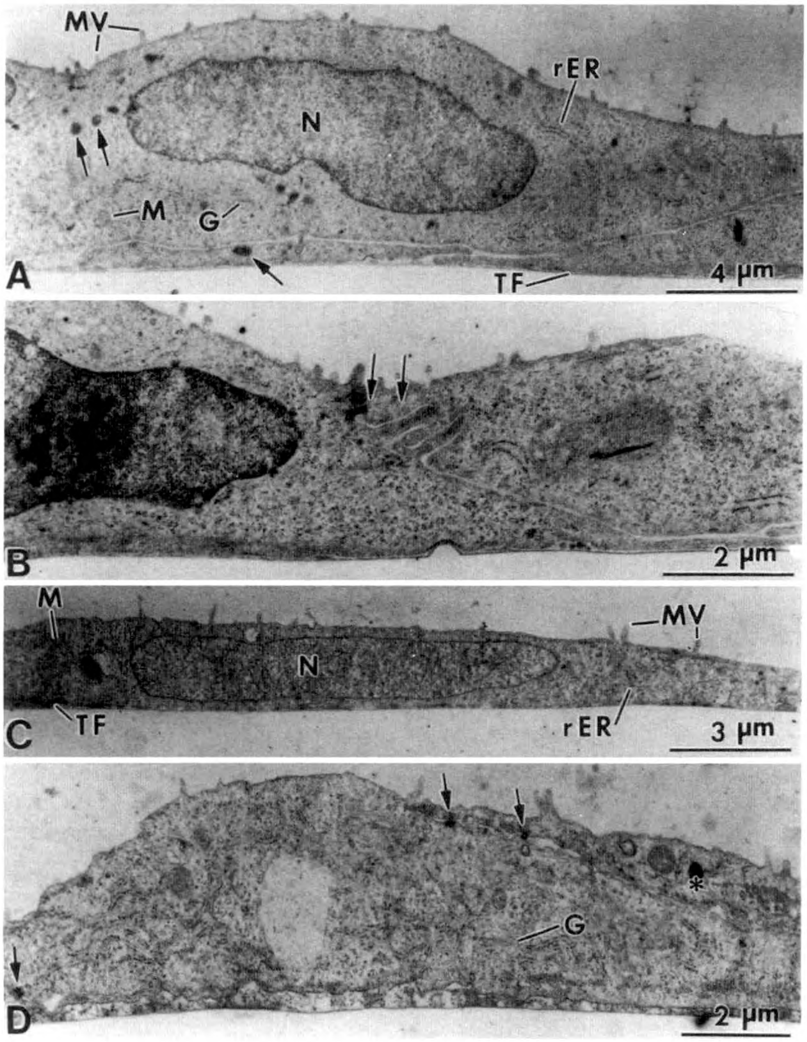


Fig 3. Transmission electron micrographs. A) Immortalized rat middle ear epithelial cells, showing large nuclei (N), well-developed rough endoplasmic reticulum (rER), numerous ribosomes, Golgi apparatus (G), microvilli (MV), mitochondria (M), and tonofilaments (TF; original ×4,000). Many lysosomal granules were observed (arrows). B) Mature junctional complexes (arrows) are shown (original ×8,000). C) Primary culture of rat middle ear epithelial cells, showing large ovoid nucleus (N), mitochondria (M), well-developed rough endoplasmic reticulum (rER), numerous ribosomes, microvilli (MV), and tonofilaments (TF; original ×5,000). D) Golgi apparatus (G) and desmosomes (arrows) are shown (original ×6,700). Lysosomal granules are also seen (asterisk).

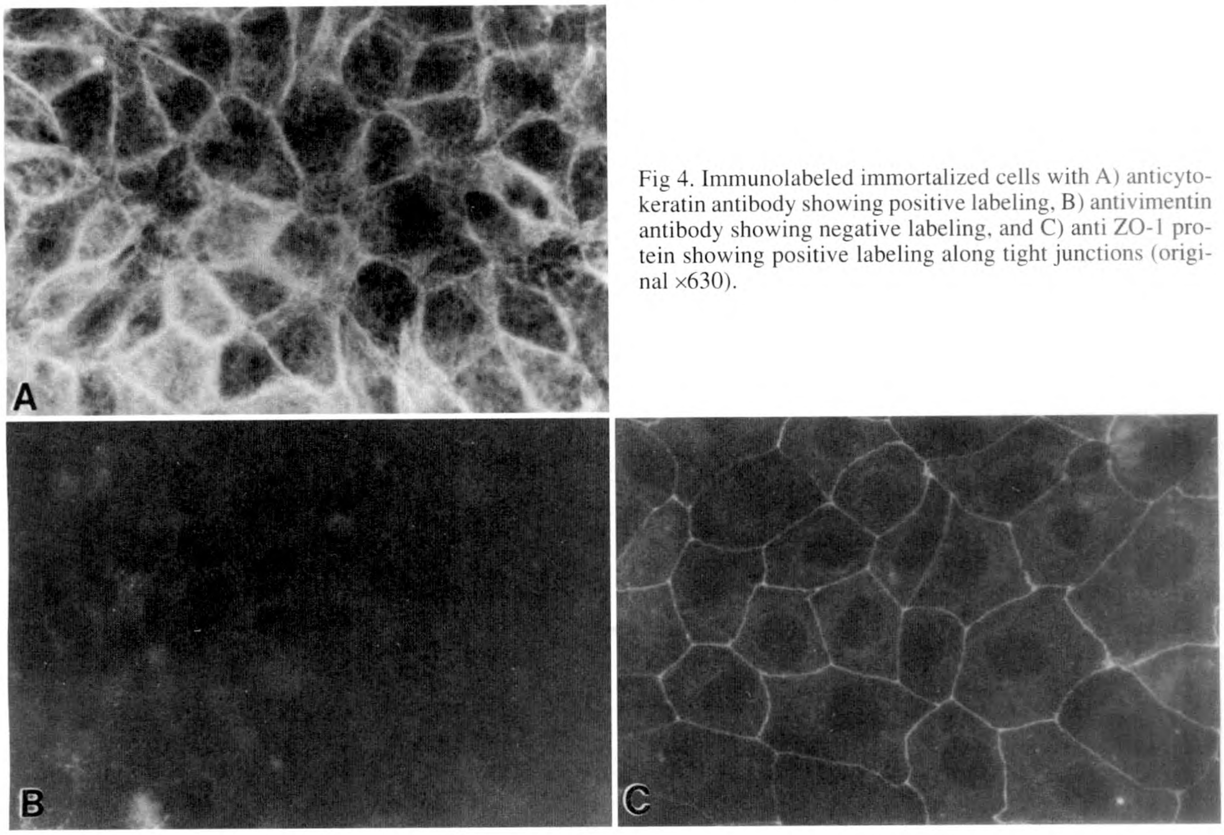


Fig 4. Immunolabeled immortalized cells with A) anticytokeratin antibody showing positive labeling, B) antivimentin antibody showing negative labeling, and C) anti ZO-1 protein showing positive labeling along tight junctions (original ×630).

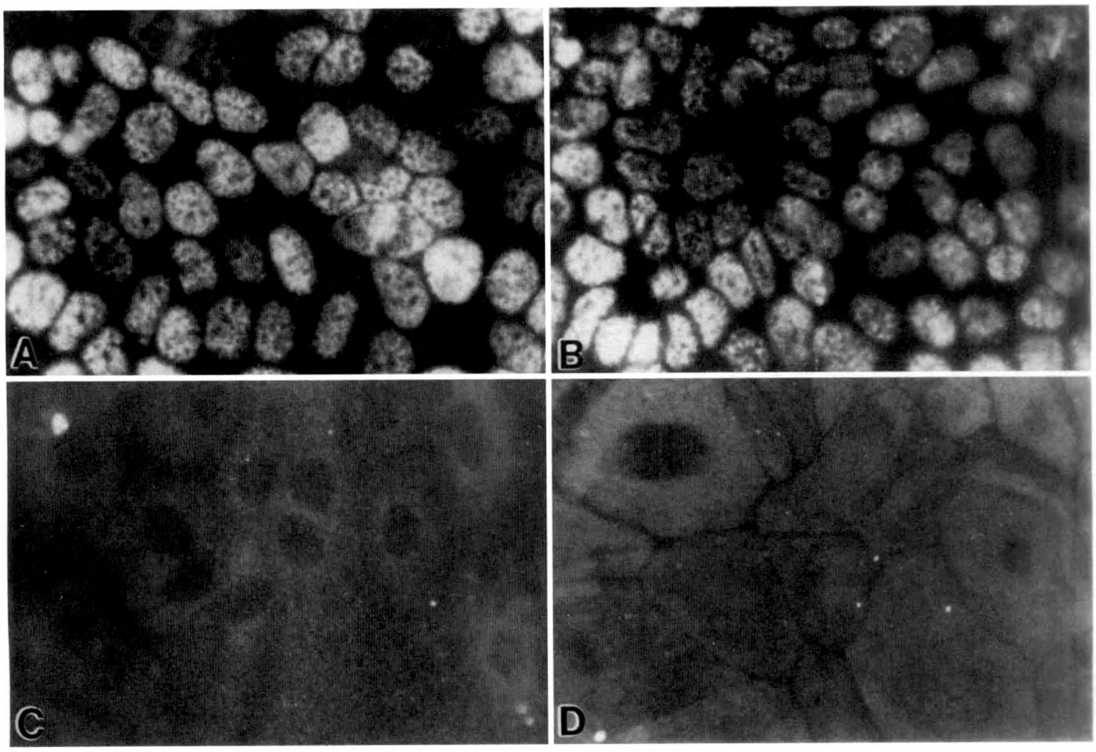


Fig 5. A,B) Immortalized cells immunolabeled with A) anti-SV40 T antigen antibody and B) anti-p53 antibody and C,D) primary cultured cells immunolabeled with C) anti-SV40 T antigen antibody and D) anti–p53 protein antibody (original ×630). Expression of SV40 T antigen and p53 protein immunolabeling was positive in nuclei of immortalized cells, but not in primary cultured cells.

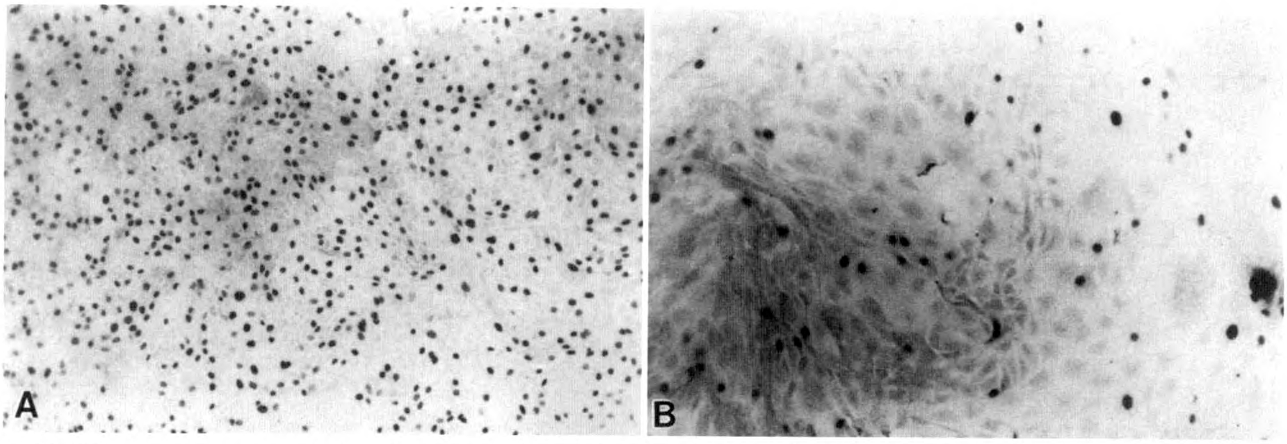


Fig 6. Bromodeoxyuridine labeling of A) immortalized cells and B) primary cells, indicating higher rate of cell proliferation in immortalized cells than in primary cells (original ×100).

immortalized cells are as follows: antibodies LDS-68, CK-E3, CKB1, K4.62, CY-90, C-11, and K8.13 and a mixture of C-11, PCK-26, CY-90, KS-1A3, M20, and A53-B/A2 labeled primary cultured cells. Antibodies 6B10, K8.60, K8.12, and PCK-26 produced no immunoreactivity in primary cultured cells. The cytokeratin expression pattern of immortalized cells was the same as that of primary cultured cells. A positive immunoreaction with monoclonal cytokeratin antibody C-11 indicated that the cell line was epithelial in nature[22-24] (Fig 4A). Vimentin staining was used to demonstrate that this cell line was not of fibroblast lineage (Fig 4B). ZO-1, a specific protein expressed in epithelial cell tight junctions,[26] appeared positive in 100% of the cells (Fig 4C).

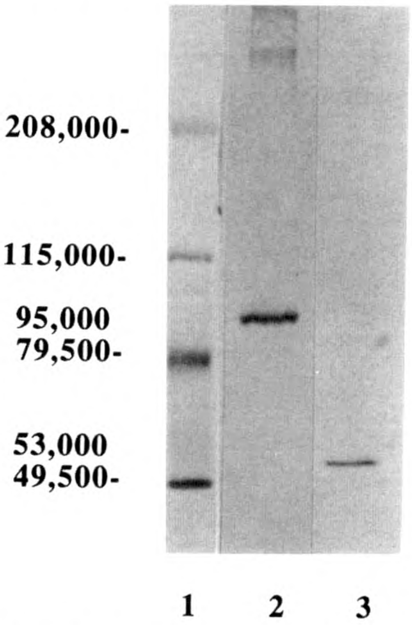


Fig 7. Western immunoblot analysis of immortalized cells. Lane 1 — markers; lane 2 — immunoblot with antibody to SV40 T antigen showing single band around 95 kd; lane 3 — immunoblot with antibody to p53 protein showing single band around 53 kd.

Intense nuclear staining in the RMEE-1 cell line was observed with antibodies against SV40 T antigen, but not in the primary cultured rat middle ear cells (Fig 5A,C). Similarly, there was a strong reaction with antibody against p53 in this cell line, but not in the primary cultured rat middle ear cells (Fig 5B,D).

BrdU Labeling. BrdU labeling showed a dramatic difference in the number of primary cultured cells and immortalized cells (RMEE-1) showing mitotic activity. The cell proliferation activity of the immortalized cells was greater than that of the primary cells (Fig 6).

Western Blot Analysis. The appearance of a single band around 90 kd is consistent with the expression of the SV40 large T antigen in immortalized cells. An anti-p53 antibody labeled a single band around 53 kd. The findings are also consistent with the expression of the p53 protein in the RMEE-1 cells (Fig 7).

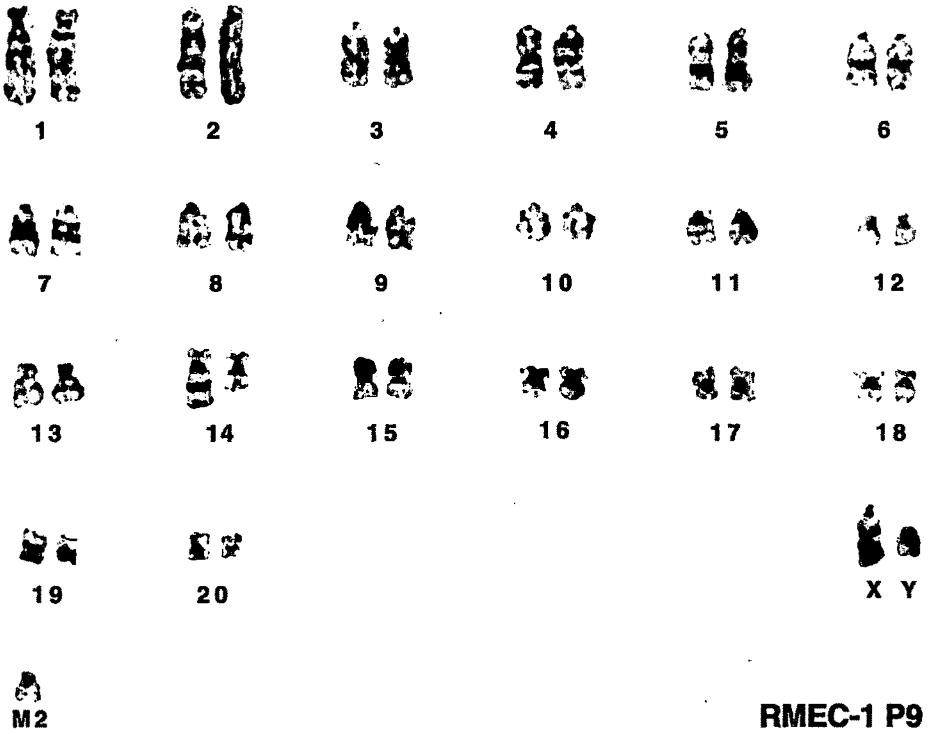
Chromosomal Analysis. Karyotypic analysis of the cell line (RMEE-1) indicated that it is composed of normal diploid rat cells with random chromosomal changes (Fig 8). Additionally, these cells exhibited signs of immortalization without expressing a transformed phenotype, as they could not grow on soft agar.

Growth Rate. The infected epithelial cells were found to have an average doubling time of 50.5 hours. In comparison, the primary cultured cells doubled every 197.3 hours (Fig 9).

DISCUSSION

The normal biology of the middle ear epithelium, such as the life cycle, cell lineage determination (secretory cell, ciliated cell), water and ion transport, secretory regulation, and ciliogenesis, is poorly understood. In otitis media, bacterial interaction with

Fig 8. Karyotypic analysis of immortalized cells. Immortalized cells are composed of normal diploid rat cells with random chromosomal changes.



middle ear epithelial cells is an important first step in the development of otitis media.[27-29] The interaction between bacterial ligands and host cell surface receptors and cell signaling pathways on the molecular level in the pathogenesis of otitis media is poorly understood.

In order to study the normal biology of the middle ear epithelial cells and better understand the cellular and molecular mechanisms of the development of otitis media, several successful procedures have been established for primary culture of middle ear epithelial cells in animals such as rats,[1] guinea pigs,[2] gerbils,[3] and chinchillas.[5-7] In addition, a gerbil middle ear epithelial cell line has been developed and examined for vectoral electrolyte transport.[8]

In order to overcome the difficulty of harvesting large quantities of cultured cells without sacrificing many animals, we have successfully immortalized rat middle ear epithelial cells using adeno 12–SV40 hybrid virus. The adeno 12–SV40 hybrid virus has been used to transform several cell types.[9-11] This virus is a hybrid of adenovirus 12 and simian virus 40 (SV40), which induces messenger RNA from the transforming region of SV40 (and not the early I region of adenovirus 12) during the transformation events.[29] It has been reported that the cell lines immortalized by this virus have been shown to be nontumorigenic, with an undifferentiated morphological phenotype.[30] We demonstrated these characteristics in our immortalized cells by showing that the cells are unable to grow on soft agar.[31] Additionally, karyotype analysis revealed that this cell line is from normal male diploid rat cells. These results indicate that this cell line does not express a fully transformed phenotype by immortalization. Positive immunoreactivity to SV40 large antigen and p53 indicates the incorporation and expression of these viral genes in the RMEE-1 cell line. These results are consistent with the results of the Western immunoblotting procedure.

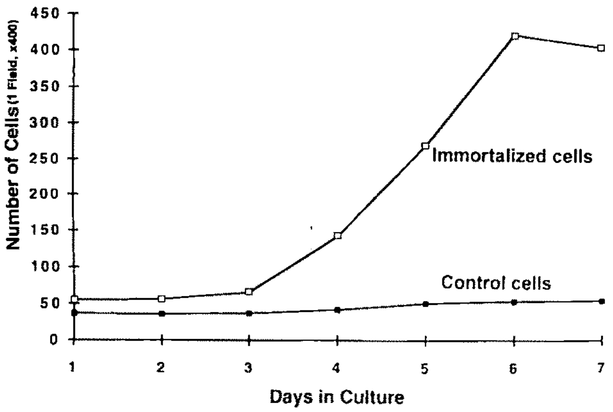


Fig 9. Numbers (per high-power field) of immortalized cells and primary culture (control) cells 1 to 7 days after plating on collagen-coated petri dish.

The data presented suggest that the RMEE-1 cell line has retained intrinsic characteristics of primary cultured epithelial cells. "Dome" formation was frequently observed by light microscopy in both the primary and the immortalized rat middle ear epithelial cells. Dome formation indicates that a monolayer of cultured epithelial cells was elevated by the accumulation of fluid under the epithelium. This finding suggests the existence of active ion and fluid transport mechanisms in these cells,[6-8] thereby suggesting that this cell line is a polarized functioning epithelial cell.

The ultrastructural findings in the RMEE-1 cells showed a similar appearance between the immortalized cells and the primary cultured cells. Both cells retain the same characteristic monolayer of epithelium with a hexagonal appearance and tight junctions. However, the immortalized cells are considerably smaller than the primary cultured cells. A TEM examination revealed the existence of tonofilaments and desmosomes in the immortalized cells, which are characteristic of primary cultured epithelial cells.[32] Both SEM and TEM analyses show that the immortalized cells retain the characteristic features of epithelial cells after immortalization. The immortalized cells stained positive for cytokeratin, but negative for vimentin. Also, ZO-1 protein was found around the border of the immortalized cells. Cytokeratins are intermediate-sized filaments containing keratin-like proteins and are found specifically in epithelial cells. Cytokeratin staining patterns in cells are completely different between species and organs and in pathological states. All monoclonal antibodies used in this experiment included polypeptides from No. 1 to No. 19, except Nos. 2, 3, 9, and 12. Among these polypeptides, Nos. 7, 14, 17, 18, and 19 were demonstrated in both the primary cultured and immortalized cells. It has been reported[14] that the simple middle ear epithelium in the chinchilla displays several polypeptides of cytokeratin, including at least Nos. 7 and 18 and possibly others. Cat middle ear epithelium demonstrated a broader immunoreactivity for cytokeratin polypeptides. In this present study, we have added a broad spectrum of antibodies to polypeptide Nos. 2, 3, 4, 5, 6, 9, 12, 14, and 17. Our rat middle ear epithelial cells showed a moderately broad range of immunoreactivity to various polypeptides. Further, this cell line (RMEE-1) retained cytokeratin expression patterns similar to those in the primary cultured epithelial cells. These results suggest that this cell line originated from middle ear epithelial cells and possesses the characteristics of epithelial cells, expressing a tight junction protein.[26]

BrdU staining shows significantly active proliferation of immortalized cells in comparison to that of primary cultured cells. This finding is consistent with the much shorter doubling time of immortalized cells in comparison to that of primary cultured cells. This cell line has demonstrated a robust cell proliferation for more than 17 months and 40 passages. This extended life and active proliferation are sufficient to generate a large number of cells for future research.

In conclusion, the immortalized rat middle ear epithelial cell (RMEE-1) possesses the features and immunohistochemical characteristics of epithelial cells. The cell line also retains features of primary cultured cells, as reflected in ultrastructural findings, chromosomal analysis, and cytokeratin staining patterns. The availability of a rat middle ear epithelial cell line will be useful for studying the normal biology of middle ear epithelial cells, including cell differentiation, ion transport, and secretory regulation, and for the elucidation of the pathogenesis of otitis media at the cellular and molecular levels.

ACKNOWLEDGMENTS — The authors gratefully acknowledge Marianne Parrakal for her technical assistance, and Dr Doris Wu and Dr Daniel Choo for their helpful criticism. Additionally, we thank Dr Valerie Dodane for providing the necessary antibody for ZO-1. We are also indebted to Shoshanna Tenn and Safura Babu-Khan for their invaluable assistance in manuscript preparation, and Gail Poulson for word processing.

REFERENCES

1. Van Blitterswijk CA, Ponec M, Van Muijen GNP, Wijsman MC, Koerten HK, Grote JJ. Culture and characterization of rat middle ear epithelium. Acta Otolaryngol (Stockh) 1986; 101:453-66.

2. Takeno S, Wake M, Harada Y, Hirakawa K, Suzuki M. Tissue culture of middle ear epithelium using fibroblast-reorganized collagen gels. J Otolaryngol 1993;22:380-4.

3. De Serres LM, Michael R, Scott V, Pillsbury HC, Prazma J. Bioelectric properties of gerbil middle ear epithelia. Arch Otolaryngol Head Neck Surg 1991;117:416-21.

4. Herman P, Friedlander G, Tran Ba Huy P, Amiel C. Ion transport by primary cultures of Mongolian gerbil middle ear epithelium. Am J Physiol 1992;262:F373-F380.

5. Nakamura A, DeMaria TF, Lim DJ, van Blitterswijk CA. Primary culture of chinchilla middle ear epithelium. Ann Otol Rhinol Laryngol 1991;100:774-82.

6. Nakamura A, DeMaria TF, Lim DJ, Arya G, van Blitterswijk C. Serial culture and characterization of the chinchilla middle ear epithelium. Ann Otol Rhinol Laryngol 1991;100: 1024-31.

7. Amesara R, Kim Y, Sano S, Harada T, Juhn SK. Primary cultures of middle ear epithelial cells from chinchillas. Eur Arch Otorhinolaryngol 1992;249:164-7.

8. Herman P, Cassigena R, Friedlander G, et al. Middle ear cell line that maintains vectorial electrolyte transport. J Cell Physiol 1993;154:615-22.

9. Lee MS, Garkovenko E, Yun JS, et al. Characterization of adult human prostatic epithelial cells immortalized by polybrene-induced DNA transfection with a plasmid containing an origin-defective SV40 genome. Int J Oncol 1994;4:821-30.

10. Andley UP, Rhim JS, Chylack LT, Fleming TP. Propagation and immortalization of human lens epithelial cells in culture. Invest Ophthalmol Vis Sci 1994;35:3094-102.

11. Arora N, Min KU, Costa JJ, Rhim JS, Metcalfe DD. Immortalization of mouse bone marrow–derived mast cells with Ad12-SV40 virus. Int Arch Allergy Immunol 1993;100:319-27.

12. Zeitlin PL, Rhim JS, Cutting G, et al. A cystic fibrosis

bronchial epithelial cell line: immortalization by adeno-12-SV40 infection. Am J Respir Cell Mol Biol 1991;4:313-9.

13. Leube RE, Bader BL, Bosch FX, Zembelmann R, Achtstaetter T, Franke WW. Molecular characterization and expression of the stratification-related cytokeratins 4 and 15. J Cell Biol 1988;106:1249-61.

14. Sano S, Schachern PA, Haruna S, Paparella MM. Cytokeratin patterns of normal middle ear epithelia in humans, cats, and chinchillas. Ann Otol Rhinol Laryngol 1994;103:227-34.

15. Caselitz J, Walther B, Wüstrow J, Seifert G, Weber K, Osborn M. A monoclonal antibody that detects myoepithelial cells in exocrine glands, basal cells in other epithelia and basal and suprabasal cells in certain hyperplastic tissues. Virchows Arch [Pathol Anat] 1986;409:725-38.

16. Troyanovsky SM, Guelstein VI, Tchipysheva TA, Krutovskikh VA, Bannikov GA. Patterns of expression of keratin 17 in human epithelia: dependency on cell position. J Cell Sci 1989; 93:419-26.

17. Lifschitz-Mercer B, Fogel M, Kushnir I, Czernobilsky B. Chorangioma: a cytoskeletal profile. Int J Gynecol Pathol 1989;8:349-56.

18. Gigi-Leitner O, Geiger B, Levy R, Czernobilsky B. Cytokeratin expression in squamous metaplasia of the human uterine cervix. Differentiation 1986;31:191-205.

19. Bartek J, Bartkova J, Papadimitriou JT, et al. Differential expression of keratin 19 in normal human epithelial tissues revealed by monospecific monoclonal antibodies. Histochem J 1986;18:565-75.

20. Huszar M, Gigi-Leitner O, Moll R, Franke WW, Geiger B. Monoclonal antibodies to various acidic (type I) cytokeratins of stratified epithelia. Differentiation 1986;31:141-53.

21. Lane EB, Rugg EL, Navsaria H, et al. A mutation in the conserved helix termination peptide of keratin 5 in hereditary skin blistering. Nature 1992;356:244-6.

22. Bartek J, Vojtesek B, Staskova Z, et al. A series of 14 new monoclonal antibodies to keratins: characterization and value in diagnostic histopathology. J Pathol 1991;164:215-24.

23. Bartkova J, Bartek J, Lukas Z, et al. Effects of tissue fixation conditions and protease pretreatment on immunohistochemical performance of a large series of new anti-keratin monoclonal antibodies: value in oncopathology. Neoplasma 1991;38:439-46.

24. Lane EB, Alexander CM. Use of keratin antibodies in tumor diagnosis. Semin Cancer Biol 1990;1:165-79.

25. Sasaki K, Ogino T, Takahashi M. Immunological determination of labeling index on human tumor tissue sections using monoclonal anti-BrdUrd antibody. Stain Technol 1986;61:155-61.

26. Stevenson BR, Siliciano JD, Mooseker MS, Goodenough DA. Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. J Cell Biol 1986;103:755-66.

27. Lim DJ, DeMaria TF, Bakaletz LO. Functional morphology of the tubotympanum related to otitis media: a review. Am J Otol 1987;8:385-9.

28. Hando S, Giebink GS. Adherence of different *Streptococcus pneumoniae* serotypes to chinchilla middle ear and tracheal epithelial cells. In: Mogi G, Honjo I, Ishii T, Takasaka T, eds. Recent Advances in Otitis Media. Proceedings of the 2nd Extra-ordinary International Symposium. Amsterdam, the Netherlands: Kugler Publications, 1994:613-8.

29. Rhim JS, Jay G, Arnstein P, Prince FM, Sandord KK, Aaronson SA. Neoplastic transformation of human epidermal keratinocytes by AD12-SV40 and Kirsten sarcoma viruses. Science 1984;227:1250-2.

30. Jambrosic J, Mancianti ML, Ricciardi R, Sela BA, Joprowski H, Jerlyn M. Transformation of normal human melanocytes and non-malignant nevus cells by adenovirus 12–SV40 hybrid virus. Int J Cancer 1989;44:1117-23.

31. Scholte BJ, Kansen M, Hoogeveen AT, et al. Immortalization of nasal polyp epithelial cells from cystic fibrosis patients. Exp Cell Res 1989;182:559-71.

32. Hanamure Y, Deguchi K, Ohyama M. Ciliogenesis and mucus synthesis in cultured human respiratory epithelial cells. Ann Otol Rhinol Laryngol 1994;103:889-95.

Ann Otol Rhinol Laryngol 110:2001

LONG-TERM STUDY OF ENDONASAL DURAPLASTY AND REVIEW OF THE LITERATURE

BERNHARD SCHICK, MD
HOMBURG, GERMANY
DOMINIK BRORS, MD
FULDA, GERMANY

RAINER IBING
FULDA, GERMANY
WOLFGANG DRAF, MD, FRCS
FULDA, GERMANY

Many different techniques have been proposed to repair frontobasal dura mater lesions. Because of its low morbidity and high success rate, the endonasal approach has become a preferred route for treating cerebrospinal fluid fistulas of the anterior skull base. This article presents a retrospective evaluation of 136 endonasal duraplasties (126 patients) performed between July 1980 and May 1998 at a tertiary care facility. Follow-up consisted of clinical examinations including nasal endoscopy, objective measures, and telephone interviews. The following measures were used to evaluate the results of these duraplasties: postoperative nasal fluorescein endoscopy in 71 cases, computed tomographic cisternography in 2, nasal fluorescein endoscopy with computed tomographic cisternography in 19, magnetic resonance imaging in 6, and nasal fluorescein endoscopy with magnetic resonance imaging in 1. Tight closure was accomplished in 129 dural lesions (94.9%) on the first attempt. In 3 cases, recurrence of cerebrospinal fluid leakage was treated successfully by 1 endonasal revision, and in 1 case, a tight duraplasty was achieved after 2 endonasal revisions. Its high success rate, low rate of morbidity, and good long-term results recommend endonasal duraplasty as a primary treatment modality for frontobasal dural lesions. For extended frontobasal dural lesions, for which intracranial dural repair is the preferred approach, the endonasal approach should be used to close additional dural leaks of the sphenoid sinus.

KEY WORDS — cerebrospinal fluid fistula, endonasal approach, skull base, surgery.

INTRODUCTION

Several techniques have been used throughout the 20th century to close cerebrospinal fluid (CSF) fistulas. In 1928, De Almeida[1] recommended endonasal cauterization of dura mater lesions with 50% chromic acid or a platinum loop as a simple way to treat CSF rhinorrhea, but 1 of the 2 patients treated by this procedure died of encephalitis 6 months later. Friedberg and Galloway[2] applied a 20% solution of silver nitrate to a CSF fistula and reported that the rhinorrhea ceased within 12 days.

The first successful surgical repair of a dural lesion, attributed to Dandy[3] in 1926, consisted of suturing a transplant of fascia lata over a dural tear behind the posterior wall of the frontal sinus via an intracranial approach. An extracranial approach for duraplasty was described by Dohlman[4] in 1948, and in 1952, Hirsch[5] reported repair of dural lesions in the sphenoid sinus via an endonasal route. In 1964, Vrabec and Hallberg[6] described the use of endonasal surgery to close CSF fistulas of the cribriform plate.

Despite these experiences with endonasal duraplasty, many otolaryngologists continued to use extracranial approaches, because the endonasal approach did not provide a sufficient view of the anterior skull base. Now, however, technical improvements in the microscope and the endoscope offer excellent visualization of the anterior skull base through the nose. In recent years, endonasal duraplasty has been used to manage a large number of dural lesions with a low rate of morbidity.[7-11] This article supports the value of endonasal duraplasty by presenting our long-term experience with the procedure and a review of the literature.

MATERIALS AND METHODS

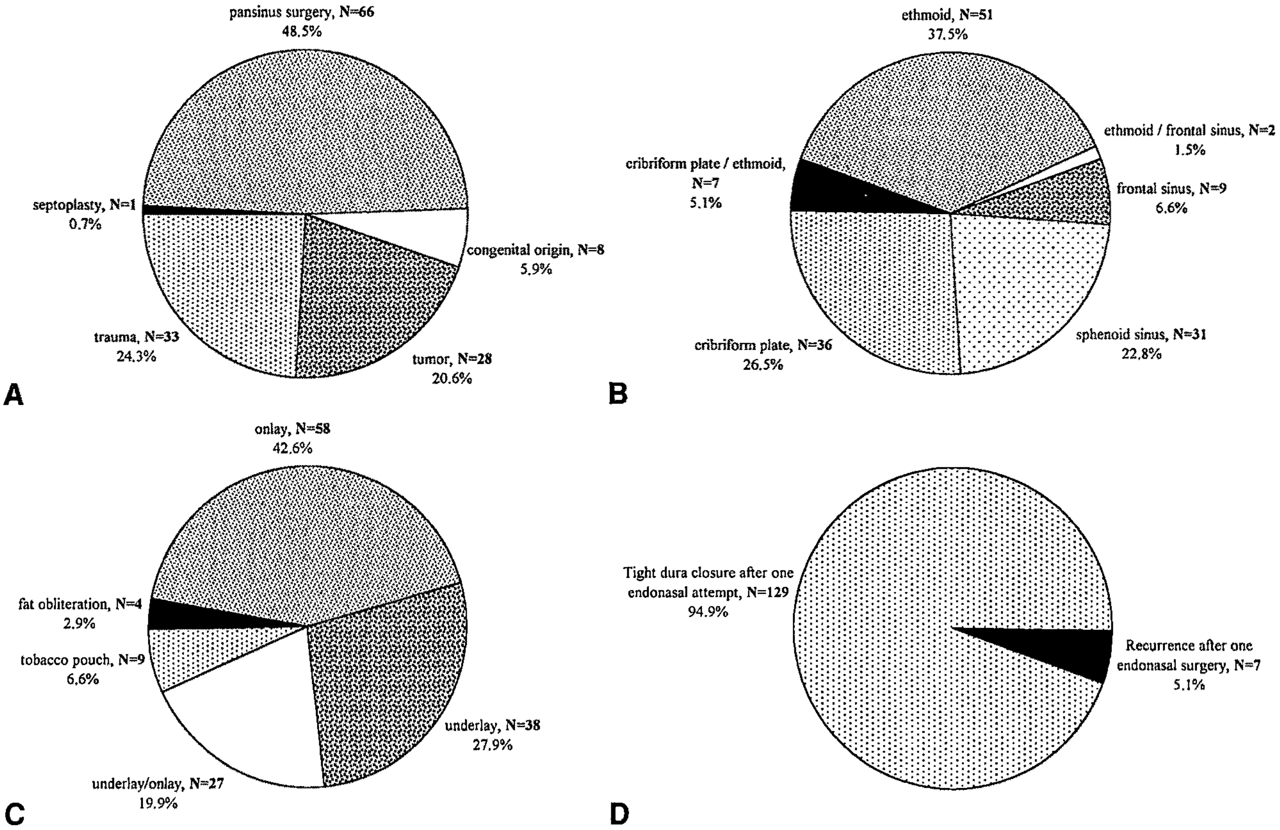
Design. Between July 1980 and May 1998, 126 patients underwent 136 duraplasty procedures by an endonasal approach at the otorhinolaryngology department of the Academic Teaching Hospital, Fulda. Information on the cause and site of the dural lesion, technique of repair, and complications were obtained from the medical reports, and follow-up data were taken from hospital records, outpatient records, and telephone interviews.

Techniques. Endonasal surgery was performed as a combined microendoscopic technique. In 41 cases of suspected CSF fistula, fluorescein was applied by lumbar puncture before surgery in order to facilitate identification of the dural lesion and to verify the duraplasty's success during the procedure. Sterile, freshly prepared 5% fluorescein solution was applied intrathecally by lumbar puncture. To prevent compli-

---

From the Department of Ear, Nose, and Throat Diseases, Head, Neck, and Facial Plastic Surgery, Communication Disorders (Schick, Brors, Draf) and Medical Clinic I (Ibing), Fulda Hospital, Academic Teaching Hospital of the University of Marburg, Fulda, Germany.

CORRESPONDENCE — Bernhard Schick, MD, ENT-Dept, University of Homburg/Saar, Kirrberger Str, D-66421 Homburg/Saar, Germany.



Data on 136 endonasal duraplasties of frontobasal dural lesions. A) Causes of lesions. B) Sites of lesions. C) Surgical techniques. D) Results on first attempt.

cations, only 0.1 mL of 5% fluorescein solution per 10 kg of body weight (but never more than 1 mL) should be used.

The onlay and underlay procedures were used as the main treatment modalities for dural repair. Whenever possible, the inserted dural graft was covered by a mucosal flap from a neighboring site in order to accelerate epithelialization. In addition, Kley's "tobacco pouch" technique (fascia lata folded like a pouch and filled with sponge tamponade to push the fascia against the osseous wall) or obliteration with abdominal fat was used to deal with dural lesions of the sphenoid sinus.[11] In 5 cases of profuse CSF rhinorrhea from the sphenoid sinus, a small piece of abdominal fat was wedged in the bony defect to reduce the CSF pressure before duraplasty. Only 1 patient required postoperative management with lumbar drainage.

Allogeneic connective tissue (Tutoplast, Biodynamics International, Erlangen, Germany) was used as graft material in 122 duraplasties. Abdominal fat and fascia lata were used as autologous grafts. Both the grafts and mucosal flaps were fixed with fibrin glue (Tissel, Immuno AG, Heidelberg, Germany).

Follow-up. In addition to clinical and endoscopic control examinations, nasal fluorescein endoscopy, computed tomographic cisternography, and magnetic resonance imaging were used after the procedure to verify a tight dural repair. The duraplasty was judged to be tight if the patients reported no watery nasal discharge or meningitis after surgery and the findings on follow-up examinations were unremarkable. Follow-up ranged from 3 to 218 months, with an average follow-up time of 67 months.

All data from the medical reports, examinations, and telephone interviews were evaluated with the SPSS statistical program (version 7.5.2G). Information selected from the medical reports and follow-up examinations was analyzed a second time to increase accuracy.

RESULTS

In 66 of the 136 endonasal duraplasties, the dural lesions occurred during paranasal sinus surgery performed to treat chronic inflammatory diseases. In addition, 33 CSF fistulas were caused by trauma, 28 dural repairs were necessary in endonasal tumor surgery with dural resection because of frontobasal tumor extension or intraoperative dural injury, 8 CSF fistulas were considered to be of congenital origin, and 1 dural fistula repair was required after septoplasty (see Figure, A). The dural lesions were located at the posterior wall of the frontal sinus in 9 cases

TABLE 1. FAILURES OF ENDONASAL DURAL REPAIR IN SERIES OF 136 ENDONASAL DURAPLASTIES

	Site	*Cause*	*Technique*	*Cause of Failure*	*Treatment*
1	Ethmoid roof	Accidental CSF fistula during sinus surgery	Onlay	Graft displacement	Endonasal revision with successful dural repair
2	Cribriform plate	Congenital CSF fistula	Onlay	Incomplete exposure of CSF fistula during 2 endonasal attempts	Tight duraplasty was accomplished after 2 endonasal revisions
3	Sphenoid sinus	Trauma	Fat obliteration	High CSF pressure	Successful revision wedging abdominal fat graft directly into fracture gap
4	Sphenoid sinus	Trauma	Fat obliteration	High CSF pressure (closure of sphenoid CSF leak was indicated because of extensive CSF loss, but worse condition of patient prevented further surgical treatments)	No revision; patient died 10 days after severe trauma due to cardiovascular shock
5	Sphenoid sinus	Tumor	Onlay	Postoperative chemotherapy in case of osteosarcoma may have contributed to insufficient dural repair	Successful endonasal revision
6	Sphenoid sinus	Tumor	Fat obliteration	Tumor recurrence	Tumor resection in neurosurgery department with successful duraplasty
7	Sphenoid sinus	Congenital CSF fistula	"Tobacco pouch"	Not definitely answered (perhaps high CSF pressure disturbed wound healing process)	After 3 intracranial and 3 extracranial attempts, 2 endonasal duraplasties failed to close CSF fistula

CSF — cerebrospinal fluid.

(6.6%), the cribriform plate in 36 (26.5%), the ethmoid roof in 51 (37.5%), and the sphenoid sinus in 31 (22.8%). In 9 cases, 2 areas were affected: in 7 cases, the cribriform plate and ethmoid, and in 2 cases, the ethmoid and posterior wall of the frontal sinus (see Figure, B).

The underlay technique was chosen for 38 cases (27.9%), and the onlay procedure was performed in 58 cases (42.6%; see Figure, C). A combination of the two techniques was used in 27 (19.9%) of the duraplasties. Nine CSF fistulas of the sphenoid sinus (6.6%) required the tobacco pouch technique, and obliteration of the sphenoid sinus with abdominal fat was accomplished in another 4 cases (2.9%). In 7 patients, the endonasal duraplasty was combined with external approaches, either because of extended frontobasal skull base fractures or because additional dural lesions were not reachable endonasally (dural leak behind the cranial part of the posterior frontal sinus wall).

One patient developed postoperative meningitis during the hospital stay, but recovered completely after antibiotic treatment. In 1 patient, the onlay graft was displaced while crusts were being cleaned from the ethmoid, but the patient did not experience a CSF leak recurrence, because a combined underlay-onlay technique had been performed in this case. Temporary headaches due to lumbar puncture were observed after fluorescein application, but there were no severe complications resulting from endonasal duraplasty or fluorescein application.

A number of objective measures were performed 4 to 12 months after endonasal duraplasty (median time, 4 months). In 71 cases, postoperative nasal fluorescein endoscopy (NFE) showed no evidence of a dural leak. Computed tomographic cisternography, used alone after 2 duraplasties and in combination with NFE in 19 cases, revealed no sign of CSF leakage. Magnetic resonance imaging, used alone in 6 cases and combined with NFE in 1 case, also showed no indication of insufficient duraplasty. One hundred two of the 126 patients were interviewed by phone and reported no watery nasal discharge or episodes of meningitis; 4 patients who had died by the time of the phone calls had had no reported signs of endocranial complications.

A tight endonasal dural repair failed after the first intervention in 7 of the 136 duraplasties (see Figure, D). The causes of these failures and their treatments are enumerated in Table 1.

DISCUSSION

Frontobasal dural lesions must be closed, because patients are at risk for potentially fatal endocranial complications such as meningitis, encephalitis, and

TABLE 2. REVIEW OF LITERATURE ON ENDONASAL DURAPLASTY

Authors	*Year*	*No. of Patients*	*Primary Success Rate*	*Follow-up*	*Comments*
Hirsch[5]	1952	3	0/3 (0%)	6 wk to 2 y	First case, intracranial revision; second case, 6 attempts; third case, 1 attempt, but after removal of nasal packing, there was still CSF leakage, which ceased within days
Vrabec and Hallberg[6]	1964	1	1/1 (100%)	22 mo	
Lehrer and Deutsch[12]	1970	2	2/2 (100%)	29 mo	Two more cases of endonasal dural repair enumerated in comment, with 1 failure
Papay et al[13]	1989	4	4/4 (100%)	6-12 mo	
Mattox and Kennedy[14]	1990	7	6/7 (86%)		Successful transnasal revision in 1 case
Stankiewicz[15]	1991	6	6/6 (100%)	17 mo (3 mo to 2 y)	
Amedee et al[16]	1993	22	21/22 (95%)	36 mo	Successful endonasal revision in 1 case
Handley et al[17]	1993	1	1/1 (100%)	18 mo	Two previous intranasal attempts had failed
Zeitouni et al[18]	1994	4	3/4 (75%)	15 mo	Three times, endonasal duraplasty as revision after prior extranasal attempts
Anand et al[19]	1995	16	15/16 (94%)	2.8 y (1-8 y)	One recurrence after 6 mo
Coiffier et al[20]	1995	6	5/5 (100%)	2 y	In 1 patient, CSF leakage could not be confirmed
Hartwein and Toepfner[21]	1995	26	26/26 (100%)	Time period of 8 years	
Yoon et al[22]	1995	8	7/8 (88%)	1.6 y (1-3 y)	One revision with proof of 2 additional CSF leaks while primary graft was well attached
Burns et al[7]	1996	42	35/42 (83%)	5-68 mo	Successful transnasal revision in 3 cases
Fronz and Wielgosz[23]	1996	5	5/5 (100%)		One case had 18-mo follow-up
Gjuric et al[8]	1996	33	52/53 (98%)*	5.6 y (1-12 y)	Despite several attempts, endonasal dural repair in 1 case not achieved
Hao[24]	1996	6	5/6 (83%)	>12 mo	One successful transnasal revision
Kelley et al[25]	1996	8	7/8 (88%)	2.3 y (1.5-4 y)	One successful dural closure achieved by endonasal revision
Lanza et al[9]	1996	36	34/36 (94%)	24.6 mo (2-57 mo)	
Lenarz et al[26]	1996	21	28/31 (90%)*		Successful revision in 3 recurrences
Milewski[27]	1996	28	26/28 (93%)	1-5 y	Successful transnasal revision in 2 cases
Sethi et al[28]	1996	5	5/5 (100%)		
Wetmore et al[29]	1996	1	1/1 (100%)	15 mo	
Jones et al[30]	1997	20	19/20 (95%)	6-36 mo	
Pia and Aluffi[31]	1997	5	5/5 (100%)	6-18 mo	
Reyt et al[32]	1997	21	17/21 (81%)	13-51 mo (14.5 mo)	Successful revision in 3 cases; 1 failure despite 2 endonasal attempts
Stammberger et al[10]	1997	35	68/72 (95%)*	19-65 mo	
Wax et al[33]	1997	8	17/18 (94%)*	3 y	
Wormald and McDonogh[34]	1997	6	6/6 (100%)	13 mo (3-18 mo)	
Donovan et al[35]	1998	8	8/8 (100%)	≥6 mo	

*Authors present combined series of endonasal and extranasal dural repair. Success rates in these series were enumerated only for whole group without mention of selected success rate for endonasal duraplasty.

cerebral abscesses. Recently, endonasal duraplasty has been recommended, because it is the least traumatic method of frontobasal dural repair, it has a high success rate, and patients retain olfaction and enjoy good long-term results.[7-11] A review of the literature on endonasal duraplasty is summarized in Table 2.[5-10,12-35]

The use of optical aids, such as the microscope and the endoscope, in endonasal surgery provides good visualization of the anterior skull base by the least traumatic route. Dural leaks of the sphenoid sinus, ethmoid roof, and cribriform plate can be accessed easily by the endonasal route. Endonasal access to CSF fistulas of the inferior-posterior frontal sinus wall medial to the lamina papyracea depends on individual anatomic characteristics, but these fistulas can be visualized and repaired if endonasal surgery succeeds in wide frontal sinus drainage. As to repair of dural lesions at the cribriform plate, if a patient's sense of smell is intact and the endonasal approach poses a high risk of damaging the olfactory fibers, the intradural approach may be the better choice.

The endonasal approach can also be used in combination with extranasal procedures. The transfrontal extradural approach is generally used in extended frontal skull base fractures to close large defects with a galea-periosteum flap, but the endonasal approach can be used to treat a CSF leak that persists at the posterior and lateral walls of the sphenoid sinus.

A number of different techniques for endonasal dural repair have been described, all of which have high success rates. In addition, a wide variety of different graft materials have been used successfully in these repairs. These include free mucosal grafts from the middle and inferior turbinates, combined mucosa-perichondrium (bone) grafts, fascia lata, abdominal fat, and muscle or allogeneic tissues. The inserted graft acts as a scaffold for the dura's wound healing process,[11] and enzymatic and cellular processes ultimately replace the graft with connective tissue. The allogeneic graft used in the presented series consists of pure extracellular collagen matrix. The high success rate in our series indicates that non–cross-linked biological grafts are as well suited as autologous grafts to obtaining tight dural closure, even in a nonsterile environment like the paranasal sinuses. However, it should be noted that the manufacturer recommends not using the material in infected areas, because colonization by bacteria may result in premature absorption of the material and thus in failure of dural repair.

Although the use of a particular technique or type of graft does not appear to be critical to the success of endonasal dural repair, certain factors are crucial in achieving a tight dural closure. Precise localization of the dural lesion and clear exposure of the site of CSF leakage are of utmost importance in securing a tight dural closure. Incomplete exposure or an unrecognized second dural defect can lead to recurrence and necessitate revision surgery. High-resolution computed tomography, magnetic resonance imaging, computed tomographic cisternography, and, especially, intraoperative NFE are the most valuable tools available for delineating a dural lesion.

After exact endonasal exposure of the dural lesion, a stable placement of the graft is required. In an animal study using a mucoperiosteum graft and an onlay technique, tight adhesion to the surrounding bone was found after 1 week.[8] A graft covering made with pedicled mucosal flaps from the surrounding area or with resorbable materials can be used to keep the graft in place until it is permanently fixed. Autologous grafts show a tendency to shrink postoperatively, so a generous amount of graft material should be used to cover the defect. Recurrence of CSF leakage in the sphenoid sinus may be caused by increased CSF pressure on the duraplasty. Wedging a piece of fat or muscle into the bony defect before graft placement is one way to ensure a stable graft placement, particularly in the sphenoid sinus.

The choice of surgical approach depends on the cause, location, and size of the dural injury. Our experiences in a series of 136 endonasal duraplasties over an 18-year period and our review of the literature demonstrate that endonasal duraplasty has a high success rate and stable long-term results. The only major side effect reported was transient hemiparesis, a rare but severe complication of endonasal duraplasty.[20] Endonasal repair is a relatively safe and effective way to close many types of frontobasal dural leaks and is recommended as a surgical approach of first choice. In extended frontobasal fractures and in dural lesions of the posterior wall of the frontal sinus that cannot be reached endonasally, an external approach is necessary. When a patient has a dural injury of the cribriform plate but has retained a sense of smell, surgeons must decide whether the endonasal approach or the intracranial approach provides the best chance of maintaining olfactory function.

REFERENCES

1. De Almeida B. Zwei Fälle von Kranio-Rhinorrhoe. Monatsschr Ohrenheilkd 1928;62:322-6.
2. Friedberg SA, Galloway TC. Spontaneous cerebrospinal rhinorrhea. Ann Otol Rhinol Laryngol 1938;47:792-4.
3. Dandy WD. Pneumocephalus (intracranial pneumocele or aerocele). Arch Surg 1926;12:949-82.
4. Dohlman G. Spontaneous cerebrospinal rhinorrhea. Acta Otolaryngol Suppl (Stockh) 1948(suppl 67):20-3.
5. Hirsch O. Successful closure of cerebrospinal fluid rhinorrhea by endonasal surgery. Arch Otolaryngol 1952;56:1-13.
6. Vrabec DP, Hallberg OE. Cerebrospinal fluid rhinorrhea by endonasal surgery. Arch Otolaryngol 1964;80:218-29.
7. Burns JA, Dodson EE; Gross CW. Transnasal endoscopic repair of cranionasal fistulae: a refined technique with long-term follow-up. Laryngoscope 1996;106:1080-3.
8. Gjuric M, Goede U, Keimer H, Wigand ME. Endonasal endoscopic closure of cerebrospinal fluid fistulas at the anterior cranial base. Ann Otol Rhinol Laryngol 1996;105:620-3.
9. Lanza DC, O'Brien DA, Kennedy DW. Endoscopic repair of cerebrospinal fluid fistulae and encephaloceles. Laryngoscope 1996;106:1119-25.

10. Stammberger H, Greistorfer K, Wolf G, Luxenberger W. Operativer Verschluß von Liquorfisteln der vorderen Schädelbasis unter intrathekaler Natriumfluoreszein-anwendung. Laryngorhinootologie 1997;76:595-607.

11. Weber R, Keerl R, Draf W, Schick B, Mosler P, Saha A. Management of dural lesions occurring during endonasal sinus surgery. Arch Otolaryngol Head Neck Surg 1996;122:732-6.

12. Lehrer J, Deutsch H. Intranasal surgery for cerebrospinal fluid rhinorrhea. Mt Sinai J Med 1970;37:133-8.

13. Papay FA, Maggiano H, Dominquez S, Hassenbusch SJ, Levine HL, Lavertu P. Rigid endoscopic repair of paranasal sinus cerebrospinal fluid fistulas. Laryngoscope 1989;99:1195-201.

14. Mattox DE, Kennedy DW. Endoscopic management of cerebrospinal fluid leaks and cephaloceles. Laryngoscope 1990; 100:857-62.

15. Stankiewicz JA. Cerebrospinal fluid fistula and endoscopic sinus surgery. Laryngoscope 1991;101:250-6.

16. Amedee RG, Mann WJ, Gilsbach J. Microscopic endonasal surgery for repair of CSF leaks. Am J Rhinol 1993;7:1-4.

17. Handley GH, Goodson MA, Real TH. Transnasal endoscopic closure of anterior fossa cerebrospinal fluid fistula. South Med J 1993;86:217-9.

18. Zeitouni A, Frenkiel S, Mohr G. Endoscopic repair of anterior skull base cerebrospinal fluid fistulas: an emphasis on postoperative nasal function maximization. J Otolaryngol 1994; 23:225-7.

19. Anand VK, Murali RK, Glasgold MJ. Surgical decisions in the management of cerebrospinal fluid rhinorrhea. Rhinology 1995;33:212-8.

20. Coiffier T, Cabanes J, Visot A, Dupuy M, Freche C, Chabolle F. Le traitement endonasal des rhinorrhées cérébro-spinales iatrogènes ou spontanées de l'étage antérieur de la base du crâne. Ann Otolaryngol Chir Cervicofac 1995;112:367-73.

21. Hartwein J, Toepfner N. The microsurgical management of CSF leaks in the anterior skull base. In: Mazzoni A, Sanna M, eds. Skull base surgery — update I. Amsterdam, the Netherlands: Kugler Publications, 1995:223-4.

22. Yoon JH, Lee JG, Kim SH, Park IY. Microscopical surgical management of cerebrospinal fluid rhinorrhea with free grafts. Rhinology 1995;33:208-11.

23. Fronz T, Wielgosz R. Die endonasale Versorgung von Liquorfisteln der Rhinobasis — Versorgung nach dem Heermann-Konzept. In: Hausamen JE, Schmelzeisen R, eds. Traumatologie der Schädelbasis. Reinbek, Germany: Einhorn-Presse Verlag, 1996:356-62.

24. Hao SP. Transnasal endoscopic repair of cerebrospinal fluid rhinorrhea: an interposition technique. Laryngoscope 1996; 106:501-3.

25. Kelley TF, Stankiewicz JA, Chow JM, Origitano TC, Shea J. Endoscopic closure of postsurgical anterior cranial fossa cerebrospinal fluid leaks. Neurosurgery 1996;39:743-6.

26. Lenarz T, Kempf HG, Ernst A. Nachweis und operative Therapie traumatischer frontobasaler Liquorfisteln. In: Hausamen JE, Schmelzeisen R, eds. Traumatologie der Schädelbasis. Reinbek, Germany: Einhorn-Presse Verlag, 1996:51-7.

27. Milewski C. Endonasale, endoskopische Therapie von Rhinobasisdefekten. In: Hausamen JE, Schmelzeisen R, eds. Traumatologie der Schädelbasis. Reinbek, Germany: Einhorn-Presse Verlag, 1996:71-4.

28. Sethi DS, Chan C, Pillay PK. Endoscopic management of cerebrospinal fluid fistulae and traumatic cephalocele. Ann Acad Med Singapore 1996;25:724-47.

29. Wetmore RF, Duhaime AC, Klausner RD. Endoscopic repair of traumatic CSF rhinorrhea in a pediatric patient. Int J Pediatr Otorhinolaryngol 1996;36:109-15.

30. Jones NS, Robertson IJA, Hughes RGM. The place of the endoscope in the management of cerebrospinal fluid rhinorrhea. Skull Base Surg 1997;7(suppl 2):31-2.

31. Pia F, Aluffi P. Trattamento microchirurgico delle fistole liquorali etmoido-sfenoidali. Acta Otorhinolaryngol Ital 1997; 17:26-31.

32. Reyt E, Righini C, Schmerber S, Colombani JM, Favre JJ, Passagia JG. Transnasal endoscopic identification and repair of cerebrospinal rhinorrhea [Abstract]. Skull Base Surg 1997;7(suppl 2):52.

33. Wax MK, Ramadan HH, Ortiz O, Wetmore SJ. Contemporary management of cerebrospinal fluid rhinorrhea. Otolaryngol Head Neck Surg 1997;116:442-9.

34. Wormald PJ, McDonogh M. "Bath-plug" technique for the endoscopic management of cerebrospinal fluid leaks. J Laryngol Otol 1997;111:1042-6.

35. Donovan DJ, Bolger W, Monacci W, Osenbach RK. Surgical management of iatrogenic encephaloceles [Abstract]. Skull Base Surg 1998;8(suppl 1):10.

Ann Otol Rhinol Laryngol 110:2001

CORRELATION BETWEEN MICROBIOLOGY AND PREVIOUS SINUS SURGERY IN PATIENTS WITH CHRONIC MAXILLARY SINUSITIS

ITZHAK BROOK, MD, MSC EDITH H. FRAZIER, MSC
BETHESDA, MARYLAND

Aspirates of 108 chronically inflamed maxillary sinuses were processed for aerobic and anaerobic bacteria. There were 295 bacterial isolates: 109 aerobic and facultative, and 186 anaerobic. The predominant aerobic isolates were *Staphylococcus aureus* (17 isolates), α-hemolytic streptococci (14), *Pseudomonas aeruginosa* (12), *Moraxella catarrhalis* (10), and *Haemophilus* spp (8). The predominant anaerobes were *Peptostreptococcus* spp (61), *Prevotella* spp (45), *Fusobacterium* spp (15), and *Propionibacterium acnes* (14). Analysis of the medical histories revealed a correlation only between the microbial results and previous sinus surgery. *Pseudomonas aeruginosa* and gram-negative aerobic bacilli (GNAB) were more often isolated in patients who had surgery (9 of 33 patients had *P aeruginosa* and 17 had GNAB) than in patients who did not have surgery (3 of 75 had *P aeruginosa* and 7 had GNAB; $p < .001$). Anaerobes were isolated more often in patients who did not have surgery (69 of 75 patients) than in those who had previous surgery (21 of 33 patients; $p < .001$). These findings illustrate the unique microbiological features of chronic maxillary sinusitis that persist after sinus surgery.

KEY WORDS — anaerobes, chronic sinusitis, *Pseudomonas aeruginosa, Staphylococcus aureus,* surgery.

INTRODUCTION

The microbiology of chronic maxillary sinusitis has been shown to be polymicrobial, consisting of aerobic and anaerobic bacteria.[1-5] The recovery rate of anaerobic bacteria varied in these studies from 25% to 56%. The prevalence of isolation of *Staphylococcus aureus* and gram-negative aerobic rods, including *Pseudomonas aeruginosa,* also varied and ranged from 1% to 29%[1-4,6,7] and from 0% to 15%,[1-4,6] respectively.

Studies have identified a higher rate of isolation of *P aeruginosa* and *S aureus* in patients with chronic sinusitis.[8,9] In one of these reports,[9] the recovery of *P aeruginosa* was more common in patients who had had prior surgery or nasal irrigation or had been on systemic steroids.

This retrospective study summarizes our experience in recovery of microorganisms in adults with chronic maxillary sinusitis, and correlates their isolation with host factors such as allergic rhinitis, asthma, systemic and topical use of steroids, previous antimicrobial therapy, and nasal irrigation.

PATIENTS AND METHODS

The study included 108 patients: 70 men and 38 women. Their ages ranged from 18 to 68 years (average, 32 years). All materials were obtained from consecutive patients who had undergone, between June 1986 and June 1995, an inferior mental antrostomy or a Caldwell-Luc operation because of chronic maxillary sinusitis. Sinusitis was judged to be present if the radiographic studies showed mucosal thickening and either an air-fluid level or complete opacification of the maxillary sinus. Occipitomental (Waters' view), lateral, oblique, and verticomental radiographs or computed tomography (CT) views were obtained. The degree of mucosal thickening was evaluated by noting the nearest distance between the air-mucosal interface and the lateral part of the sinus wall. Mucosal thickening was defined as a mucosal width of 5 mm or more. Patients had at least 1 of the following complaints: facial pain, frontal headache, purulent nasal discharge, fever, or malaise. All patients had received antimicrobial therapy in the past 3 months. Chronic sinusitis was defined as an infection of at least 3 months' duration.

Inferior meatal antrostomy was performed after disinfection of the oral mucosa with Betadine. The specimens that were collected during surgery were obtained under strict asepsis to avoid any contamination. The specimens were transported to the laboratory in a syringe sealed with a rubber stopper after evacuation of the air in the syringe. The time between the collection of materials and the inoculation of the specimen generally did not exceed 30 minutes; however, the exact time needed for transportation was not recorded.

Specimens were inoculated onto 5% sheep's blood, chocolate, and MacConkey's agar plates for aerobic and facultative organisms. The plates were incubated

---

From the Departments of Pediatrics (Brook) and Infectious Diseases (Frazier), Navy Hospital, Bethesda, Maryland. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or as reflecting the views of the Department of the Navy or the Naval Service at large.

CORRESPONDENCE — Itzhak Brook, MD, MSc, PO Box 70412, Chevy Chase, MD 20813-0412.

at 37°C aerobically (MacConkey's agar) or under 5% carbon dioxide (5% sheep's blood and chocolate agars) and examined at 24 and 48 hours. For anaerobes, the material was plated onto prereduced vitamin K1–enriched *Brucella* blood agar, an anaerobic blood agar plate containing kanamycin sulfate and vancomycin hydrochloride, an anaerobic blood plate containing colistin sulfate and nalidixic acid, and an enriched thioglycolate broth (containing hemin and vitamin K1).[10] The anaerobic plates and thioglycolate broth were incubated in jars (GasPak, Baltimore Biological Laboratories, Cockeysville, Md) and examined at 48 and 96 hours.

Anaerobes were identified by techniques previously described.[10] Aerobic bacteria were identified by conventional methods.[11] β-Lactamase activity was determined for all isolates by the chromogenic cephalosporin analogue 87/312 method.[12] Some of the microbiological results have been published before.[3]

Included in the study were only those patients whose medical records were available for review. An additional 34 patients were not included in the final analysis because their records were not available for review. The patients' records were reviewed, and the following medical histories were recorded and correlated with the microbiological results: allergic rhinitis (found in 14 patients), asthma (in 8), systemic disorders (in 3), use of systemic antibiotics within the past 3 weeks (in 52), use of systemic (in 12) or topical (in 18) steroids within the past 6 weeks, and previous sinus surgery (in 33). Statistical significance in the prevalence of recovery of bacteria was calculated by a χ^2 test with a Yates correction.

RESULTS

Aerobic or facultative bacteria alone were present in 18 cases (17%), anaerobic bacteria alone were recovered in 38 specimens (35%), and mixed aerobic and anaerobic bacteria in 52 (48%). There were 295 bacterial isolates recovered from the 108 specimens (2.7 per specimen). One hundred nine of the isolates were aerobic or facultative organisms (1.0 per specimen). The predominant aerobic or facultative organisms were *S aureus*, α-hemolytic streptococci, *P aeruginosa*, *Moraxella catarrhalis*, and *Haemophilus* species. There were 186 anaerobes isolated (1.7 per specimen). The predominant anaerobes were *Peptostreptococcus* species, *Prevotella* species, *Fusobacterium* species, *Propionibacterium acnes*, *Porphyromonas asaccharolytica*, and *Bacteroides fragilis* group.

Ninety-two β-lactamase–producing bacteria (BLPB) were isolated from 78 patients (72%; see Table). These included all isolates of *S aureus*, *M catarrhalis*, and the *B fragilis* group, 24 of 45 (53%) *Prevotella* species, 11 of 12 (92%) *P aeruginosa*, 7 of 15 *Fusobacterium* species, 3 of 11 (27%) *Porphyromonas* species, and 5 of 8 (62%) *Haemophilus* species.

Correlation between the clinical and medical histories revealed only differences related to prior sinus surgery. The rate of recovery of aerobic bacteria in patients who had previous sinus surgery was 1.21 per specimen, as compared to 0.92 per specimen in those with no prior surgery. In contrast, the rate of recovery of anaerobic bacteria was 1.39 per specimen in those who had prior surgery, compared to 1.87 per specimen in those who did not have prior surgery.

Pseudomonas aeruginosa and all gram-negative aerobic bacilli were more often recovered from patients who had prior sinus surgery. Gram-negative aerobic bacilli were isolated in 17 patients (including 9 with *P aeruginosa*) of 33 who had had previous surgery; in contrast, gram-negative aerobic bacilli were isolated in 7 patients (including 3 with *P aeruginosa*) of 75 who had had no surgery ($p < .001$, for surgery versus no surgery). Anaerobic bacteria were isolated more often in patients who had not had previous sinus surgery (69 of 75 patients) than in those who had had previous surgery (21 of 33 patients; $p < .001$).

Of the anaerobic bacteria, *P acnes* was more often recovered in patients who had had previous surgery (8 of 33) than in those who had not (6 of 75; $p < .025$), and *Peptostreptococcus* species were more often isolated in those who had not had surgery (49 of 75), than in those who had had surgery (12 of 33; $p < .001$). No other correlations were found between the medical history and microbiological findings.

DISCUSSION

This report confirms findings from previous studies that demonstrate the predominance of anaerobic organisms (pigmented *Prevotella* and *Porphyromonas*, *Fusobacterium*, and *Peptostreptococcus* species) in chronic maxillary sinusitis.[1-5] The microbiology of chronic maxillary sinusitis differs from that of acute maxillary sinusitis, in which *S pneumoniae*, *H influenzae*, and *M catarrhalis* predominate.[5] We isolated BLPB in almost three fourths of the patients. The recovery of BLPB is not surprising, since all of our patients had received antimicrobial agents, including β-lactams, within the past 3 months, and this therapy might have selected for these organisms. Also, the bacterial flora recovered from patients with chronic maxillary sinusitis who had prior sinus surgery was different from that of patients who did not undergo such surgery. These patients had a higher

BACTERIOLOGY OF 108 PATIENTS WITH CHRONIC MAXILLARY SINUSITIS

Bacteria	*Previous Surgery (N = 33)*	*No Surgery (N = 75)*	*Total (N = 108)*
Aerobic bacteria			
α-Hemolytic streptococci	4	10	14
Microaerophilic streptococci	2	6	8
Streptococcus pneumoniae	2	5	7
Streptococcus pyogenes	3	5	8
Group B streptococci	1	4	5
Group F streptococci	0	2	2
Staphylococcus aureus	5 (5)	12 (12)	17 (17)
Staphylococcus epidermidis	1 (1)	3 (1)	4 (2)
Moraxella catarrhalis	2 (2)	8 (8)	10 (10)
Haemophilus influenzae	1 (1)	4 (2)	5 (3)
Haemophilus parainfluenzae	1 (1)	2 (1)	3 (2)
Klebsiella species	1	1	2
Klebsiella pneumoniae	2 (2)	2 (1)	4 (3)
Escherichia coli	3 (2)	2 (1)	5 (3)
Pseudomonas aeruginosa	9* (8)	3 (3)	12 (11)
Stenotrophomonas maltophilia	2	0	2
Serratia marcescens	1	0	1
Subtotal for aerobes	40 (22)	69 (29)	109 (51)
Anaerobic bacteria			
Peptostreptococcus species	12*	49	61
Veillonella parvula	2	7	9
Eubacterium species	1	3	4
Propionibacterium acnes	8†	6	14
Propionibacterium avidum	2	2	4
Clostridium species	0	4	4
Fusobacterium species	2 (2)	6 (1)	8 (3)
Fusobacterium nucleatum	1 (1)	6 (3)	7 (4)
Bacteroides species	3	9	12
Bacteroides fragilis group	2 (2)	5 (5)	7 (7)
Prevotella melaninogenica	5 (3)	17 (12)	22 (15)
Prevotella oralis	2 (1)	4 (1)	6 (2)
Prevotella oris-buccae	0	3 (1)	3 (1)
Prevotella intermedia	4 (2)	10 (4)	14 (6)
Porphyromonas asaccharolytica	2 (2)	9 (1)	11 (3)
Subtotal for anaerobes	46* (13)	140 (28)	186 (41)
Total	86 (35)	209 (57)	295 (92)

Numbers within parentheses indicate β-lactamase producers.

*Statistically significant difference ($p < .001$) as compared to other group.

†Statistically significant difference ($p < .025$) as compared to other group.

rate of recovery of aerobic bacteria that was due to the higher isolation rate of aerobic gram-negative bacilli. In contrast, they had a smaller rate of recovery of anaerobic bacteria, with the exception of *P acnes*.

The frequent involvement of anaerobes in chronic sinusitis probably is related to the poor drainage and increased intranasal pressure that develop during inflammation.[13] This can reduce the oxygen tension in the inflamed sinus[14] by decreasing the mucosal blood flow[15] and depressing the ciliary action.[16] The lowering of the oxygen content and pH of the sinus cavity supports the growth of anaerobic organisms by providing them with an optimal oxidation-reduction potential.[16] The decreased frequency of recovery of anaerobic bacteria found after surgery may be due to the improved ventilation of the maxillary sinuses, which may interfere with their growth. In contrast, the increased isolation of *P acnes*, which is a known member of the skin flora[17] and a rare pathogen, in patients with surgery may be due to its ability to colonize and possibly contribute to infection in this type of patient.

We also observed, as did Nadel et al,[9] an increased likelihood of recovery of *P aeruginosa* and other aerobic gram-negative aerobic bacilli in patients who had had surgery. It is possible that postsurgical alteration in the mucosa or mucociliary function may predispose the patient to infection by these organisms.

Pseudomonas aeruginosa is a known sinus pathogen in patients with cystic fibrosis,[18] patients with nosocomial sinusitis,[19] and immunocompromised and neutropenic patients with sinusitis.[20] Some patients who are especially prone to *P aeruginosa* sinus infection are those who have nasoenteric tubes[21] or are intubated.[22] Similarly to other investigators,[9] we did not find a correlation between the isolation of *S aureus* and any other factors.

Our findings suggest that the microbiology of chronic maxillary sinusitis in patients who have had sinus surgery is uniquely different from that of patients who have not undergone surgery. The surgical patients have microbial flora that may contain gram-negative aerobic bacteria in addition to other aerobic and anaerobic bacteria. Obtaining a culture of the maxillary sinus in such patients may therefore be of particular importance, so that appropriate antimicrobial therapy directed at their specific pathogens can be administered. Prospective studies that would identify the unique bacterial flora of patients who have had sinus surgery, and the best therapies for their inflammation, are warranted.

ACKNOWLEDGMENTS — The authors acknowledge the efforts of Dr D. Thompson and the staffs of the Department of Otolaryngology and the Clinical Microbiology Laboratory Services at the Navy Hospital in Bethesda, Maryland, and the secretarial assistance of Joanie Pietrafitta.

REFERENCES

1. Frederick J, Braude AI. Anaerobic infection of the paranasal sinuses. N Engl J Med 1974;290:135-7.

2. Su W-Y, Liu C, Hung S-Y, Tsai W-F. Bacteriological study in chronic maxillary sinusitis. Laryngoscope 1983;93:931-4.

3. Brook I. Bacteriology of chronic maxillary sinusitis in adults. Ann Otol Rhinol Laryngol 1989;98:426-8.

4. Karma P, Jokipii L, Sipilä P, Luotonen J, Jokipii AMM. Bacteria in chronic maxillary sinusitis. Arch Otolaryngol 1979; 105:386-90.

5. Nord CE. The role of anaerobic bacteria in recurrent episodes of sinusitis and tonsillitis. Clin Infect Dis 1995;20:1512-24.

6. Palva T, Gronroos JA, Palva A. Bacteriology and pathology of chronic maxillary sinusitis. Acta Otolaryngol (Stockh) 1962;54:159-75.

7. Catlin FI, Cluff LE, Reynolds RC. The bacteriology of acute and chronic sinusitis. South Med J 1965;58:1497-501.

8. Bolger WE. Gram-negative sinusitis: an emerging clinical entity? Am J Rhinol 1994;8:279-84.

9. Nadel DM, Lanza DC, Kennedy DW. Endoscopically guided cultures in chronic sinusitis. Am J Rhinol 1998;12:233-41.

10. Sutter VL, Citron DM, Edelstein MAC, Finegold SM. Wadsworth anaerobic bacteriology manual. 4th ed. Belmont, Calif: Star Publishing Co, 1985.

11. Lennette EH, Balows A, Hausler W, Shadomy HJ. Manual of clinical microbiology. 4th ed. Washington, DC: American Society for Microbiology, 1985.

12. O'Callaghan DH, Morris A, Kirby SM, Shingler AH. Novel method for detection of β-lactamase by using a chromogenic cephalosporin substrate. Antimicrob Agents Chemother 1972;1:283-8.

13. Drettner B, Lindholm CE. The borderline between acute rhinitis and sinusitis. Acta Otolaryngol (Stockh) 1967;64:508-13.

14. Carenfelt C, Lundberg C. Purulent and non-purulent maxillary sinus secretions with respect to pO2, pCO2 and pH. Acta Otolaryngol (Stockh) 1977;84:138-44.

15. Aust R, Drettner B. Oxygen tension in the human maxillary sinus under normal and pathological conditions. Acta Otolaryngol (Stockh) 1974;78:264-9.

16. Carenfelt C. Pathogenesis of sinus empyema. Ann Otol Rhinol Laryngol 1979;88:16-20.

17. Brook I, Frazier EH. Infections caused by *Propionibacterium* species. Rev Infect Dis 1991;13:819-22.

18. Nishioka GJ, Cook PR. Paranasal sinus disease in patients with cystic fibrosis. Otolaryngol Clin North Am 1996;29: 193-205.

19. George DL, Falk PS, Meduri GU, et al. Nosocomial sinusitis in patients in the medical intensive care unit: a prospective epidemiological study. Clin Infect Dis 1998;27:463-79.

20. Decker CF. Sinusitis in the immunocompromised host. Curr Infect Dis Rep 1999;1:27-32.

21. Bert F, Lambert-Zechovsky N. Microbiology of nosocomial sinusitis in intensive care unit patients. J Infect Dis 1995;31: 5-8.

22. Brook I. Microbiology of nosocomial sinusitis in mechanically ventilated children. Arch Otolaryngol Head Neck Surg 1998;124:35-8.

Ann Otol Rhinol Laryngol 110:2001

LOSS OF SECONDARY ESOPHAGEAL PERISTALSIS IS NOT A CONTRIBUTORY PATHOGENETIC FACTOR IN POSTERIOR LARYNGITIS

SECKIN O. ULUALP, MD
MILWAUKEE, WISCONSIN
ROBERT J. TOOHILL, MD
MILWAUKEE, WISCONSIN
CHENGMING GU, MD
BEIJING, CHINA
REZA SHAKER, MD
MILWAUKEE, WISCONSIN

Secondary esophageal peristalsis helps prevent the entry of gastric acid into the pharynx by clearing the refluxed gastric contents back into the stomach. Because the loss of this mechanism may contribute to the pathogenesis of reflux-induced laryngeal disorders, our aim was to study the frequency of stimulation and parameters of secondary esophageal peristalsis in patients with posterior laryngitis (PL). We studied 14 patients (45 ± 5 years) with PL documented by videolaryngoscopy and 11 healthy controls (46 ± 6 years). The upper esophageal sphincter (UES) pressure was monitored by a sleeve assembly incorporating an injection port 5 cm distal to the sleeve. The esophageal body and lower esophageal sphincter (LES) pressures were measured by an LES sleeve assembly. Primary esophageal peristalsis was induced by 5-mL water swallows. Secondary esophageal peristalsis was induced by abrupt injection of volumes of air, incrementally increased by 5 mL, into the esophagus. Secondary esophageal peristalsis could not be elicited by injection of any volume (up to 60 mL) in 3 PL patients and 2 controls. These 5 subjects had normal primary peristalsis. The threshold volume of air required to stimulate secondary esophageal peristalsis in PL patients (median, 15 mL) was similar to that of controls (median, 10 mL). The parameters of the secondary esophageal peristaltic pressure wave were similar in both groups, and in both groups, they were similar to those of primary peristalsis. The UES response to the injection of the threshold volume that induced secondary esophageal peristalsis in PL patients was contraction in 58% of the trials, partial relaxation in 3%, and no response in 39%. The findings were similar to those in the controls. The LES response to injection of the threshold volume was complete relaxation in both the PL patients and the controls. We conclude that the integrity of secondary esophageal peristalsis is preserved in PL patients.

KEY WORDS — esophagus, gastroesophageal acid reflux, peristalsis, posterior laryngitis, sphincter.

INTRODUCTION

According to the mode of its initiation, esophageal peristalsis has been classified into primary (induced by swallowing) and secondary (initiated in response to local esophageal stimuli) peristalsis.[1,2] The neuromuscular mechanisms of secondary esophageal peristalsis in humans and several other species[3-10] have been studied extensively. Secondary esophageal peristalsis is generally believed to play an important role in the volume clearance from the esophagus of the ingested material left behind after a swallow, as well as of the refluxate of gastroesophageal reflux episodes. In the latter instance, in addition to its role in esophageal acid clearance, the secondary peristalsis may be regarded as one of the mechanisms that helps prevent the refluxate from reaching the airway. Previous studies have shown a significant decrease in the frequency of stimulation of secondary peristalsis in patients with gastroesophageal reflux disease[11] and in the elderly.[12] However, secondary esophageal peristalsis in posterior laryngitis patients who have suffered the consequences of gastroesophagopharyngeal reflux events has not been systematically studied. Conceivably, the loss of this reflexively stimulated peristalsis could be a contributory factor in the entry of gastric refluxate into the pharynx and its contact with the laryngeal structures. To address this issue, we determined the threshold volume of air required to stimulate secondary esophageal peristalsis in patients with posterior laryngitis, as well as the parameters of such peristalsis, and compared the findings with those of age-matched healthy controls.

METHODS

We studied 14 consecutive patients with posterior laryngitis (10 male, 4 female), ages 17 to 74 years (45 ± 5 years), and 11 healthy volunteers (7 male, 4 female), ages 23 to 76 years (46 ± 6 years). The studies were approved by the Human Research Review Committee of the Medical College of Wisconsin, and the participants gave written informed consent before their studies. All posterior laryngitis patients and healthy volunteers filled out a detailed health questionnaire before their studies.

The healthy volunteers were recruited by advertisement and did not have any esophageal or laryngeal

---

From the Medical College of Wisconsin Dysphagia Institute (all authors), the Department of Otolaryngology and Human Communication Sciences (Ulualp, Toohill, Shaker), and the Division of Gastroenterology and Hepatology (Ulualp, Gu, Shaker), Medical College of Wisconsin, Milwaukee, Wisconsin. Supported in part by National Institutes of Health grant R01 DK25731.

CORRESPONDENCE — Reza Shaker, MD, Division of Gastroenterology and Hepatology, Froedtert Memorial Lutheran Hospital, 9200 W Wisconsin Ave, Milwaukee, WI 53226.

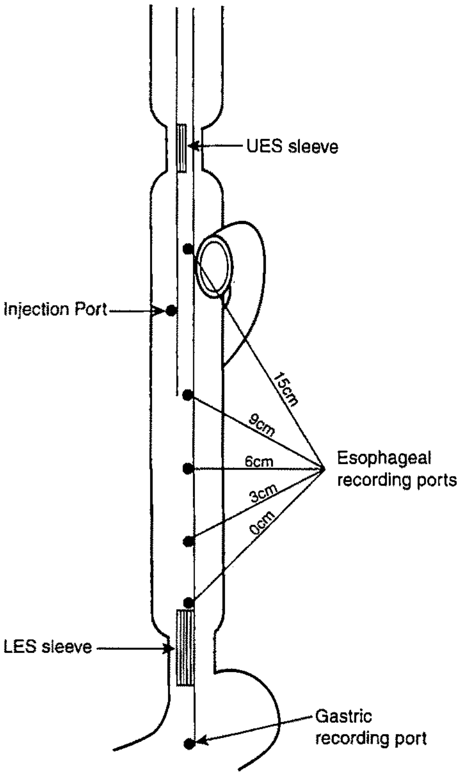


Fig 1. Schematic representation of experimental setup. UES — upper esophageal sphincter, LES — lower esophageal sphincter.

symptoms. In addition, they underwent unsedated transnasal pharyngoesophagogastroduodenoscopy and did not exhibit any esophageal abnormalities or hiatal hernia.

The patients with posterior laryngitis complained of intermittent or chronic hoarseness, frequent throat clearing, sore throat, or chronic cough and exhibited videolaryngoscopic findings of 1 or more of the following: erythema and edema of the arytenoids, erythema of the interarytenoid tissue and the posterior third of the vocal cords, pachyderma laryngis, hyperplastic interarytenoid tissue, and erythema of the entire larynx. The posterior laryngitis patients also underwent transnasal pharyngoesophagogastroduodenoscopy and did not exhibit any esophageal abnormalities or hiatal hernia. None of the studied patients or controls were taking any medication that could have potentially affected esophageal peristalsis.

To stimulate and study secondary esophageal peristalsis, we used an assembly with 2 recording catheters. One catheter incorporated an upper esophageal sphincter (UES) sleeve device (6 × 0.6 × 0.4 cm; Dentsleeve, Adelaide, Australia) and an air injection port located 5 cm distal to the lower margin of the sleeve. The other catheter incorporated a lower esophageal sphincter (LES) sleeve device (6 × 0.6 × 0.5 cm), 5 esophageal ports located 0, 3, 6, 9, and 15 cm proximal to the upper margin of the sleeve, and 1 gastric recording site. In addition, both catheters incorporated recording side holes at the proximal and distal margins of the sleeve devices for manometric positioning (Fig 1). After an overnight fast, the subjects' nasal cavities were anesthetized with 2% topical Xylocaine on a cotton-tipped applicator, and then each catheter was inserted through one nostril and positioned manometrically such that the UES sleeve straddled the UES and the LES sleeve straddled the LES. The UES and LES sleeve devices and esophageal manometric recording sites were infused with distilled water by a minimally compliant pneumohydraulic perfusion system (Arndorfer Specialties, Greendale, Wis), and the pressure tracings were recorded on a polygraph chart recorder (Grass Instruments, Quincy, Mass).

Rapid intraesophageal air injection (86 ± 3 mL/s) was used to stimulate secondary esophageal peristalsis. The onset and offset of air injection were recorded on chart paper run at a speed of 10 mm/s. To prevent stimulation of swallowing, the manometric port at the proximal margin of the UES sleeve was not perfused after positioning of the assembly.

The subjects were studied in the supine position after the UES and LES sleeves were positioned. The primary esophageal peristaltic wave was evaluated by 5-mL water swallows. For stimulation of secondary peristalsis, we started the air injections with 5 mL of air and then increased the volume by 5-mL increments until a secondary esophageal peristaltic wave was observed or the volume of injected air reached 60 mL. The subjects were instructed to withhold their swallows during the postinjection period for 15 to 20 seconds. This was followed by a "dry swallow" performed to clear any residual air. Each air injection was done at least 15 to 20 seconds after a primary peristaltic wave due to the dry swallow reached the LES, and was done 3 times. The threshold volume was determined as the lowest injected air volume that triggered the secondary esophageal peristaltic pressure wave in all 3 trials.

Using these techniques in each subject, we determined 1) the lowest injected air volume that triggered the secondary esophageal peristalsis in 3 consecutive trials (threshold volume) and 2) the amplitude, duration, and velocity of the primary and secondary esophageal peristaltic pressure waves.

Statistical comparisons between groups were performed by a 1-way analysis of variance or a Kruskal Wallis 1-way analysis of variance on ranks, and within-group comparisons were performed by a paired *t*-test or a Wilcoxon signed rank test as appropriate.

TABLE 1. COMPARISON OF CONTROLS AND POSTERIOR LARYNGITIS PATIENTS IN TERMS OF PARAMETERS OF PRIMARY AND SECONDARY ESOPHAGEAL PERISTALSIS AT VARIOUS RECORDING SITES

	Intraesophageal Recording Site									
	0 cm		*3 cm*		*6 cm*		*9 cm*		*15 cm*	
Parameter	*PP*	*SP*	*PP*	*SP*	*PP*	*SP*	*PP*	*SP*	*PP*	*SP*
Amplitude (mm Hg)										
Patients	64 ± 8	76 ± 13	88 ± 6	101 ± 11	86 ± 6	96 ± 12	76 ± 9	72 ± 14	45 ± 7	48 ± 7
Controls	93 ± 17	79 ± 16	99 ± 21	82 ± 9	92 ± 21	66 ± 8	106 ± 21	71 ± 10	76 ± 7	50 ± 5
Duration (s)										
Patients	3.1 ± 0.6	4.0 ± 0.4	2.5 ± 0.4	3.7 ± 0.3	2.3 ± 0.3	2.9 ± 0.2	2.3 ± 0.3	3.4 ± 0.4	2.1 ± 0.2	2.9 ± 0.2
Controls	3.4 ± 0.4	3.4 ± 0.3	3.7 ± 0.4	3.0 ± 0.2	3.5 ± 0.3	3.0 ± 0.3	3.0 ± 0.2	2.6 ± 0.1	2.6 ± 0.3	2.5 ± 0.1

PP — primary esophageal peristalsis, SP — secondary esophageal peristalsis.

Data are given as mean ± SE unless stated otherwise.

RESULTS

Primary Esophageal Peristaltic Pressure Wave. Primary esophageal peristalsis was documented in all patients and healthy controls after 5-mL water swallows. The average amplitude of the primary esophageal peristaltic pressure wave in the posterior laryngitis patients (72 ± 5 mm Hg) was similar to that of the controls (92 ± 14 mm Hg). Between-group comparisons did not show any significant difference between groups in the duration or velocity of the primary esophageal peristaltic pressure wave (Table 1).

Secondary Esophageal Peristaltic Pressure Wave. Examples of stimulation of secondary esophageal peristalsis by intraesophageal air injection are shown in Fig 2. In 3 patients (ages, 57, 63, and 72 years) and 2 healthy controls (ages, 69 and 76 years), secondary esophageal peristalsis could not be triggered by injection of any volume up to 60 mL. The threshold volume of air required to trigger the secondary esophageal peristaltic wave in patients with posterior laryngitis ranged from 5 to 30 mL (median, 15 mL) and was similar to that of the controls, which also ranged between 5 and 30 mL (median, 10 mL; Fig 3). Injection of the threshold volume did not induce belching in either group. The amplitude, duration, and velocity of the secondary esophageal peristaltic pressure wave were not different between the two groups (Fig 4). In both groups, the parameters of the secondary esophageal peristaltic wave were similar to those of the primary esophageal peristalsis induced by the 5-mL water swallows.

UES and LES Response to Intraesophageal Air Injection. The UES response to the injected threshold volume of air that induced secondary esophageal peristalsis in posterior laryngitis patients was contraction in 58% of the trials, partial relaxation in 3%,

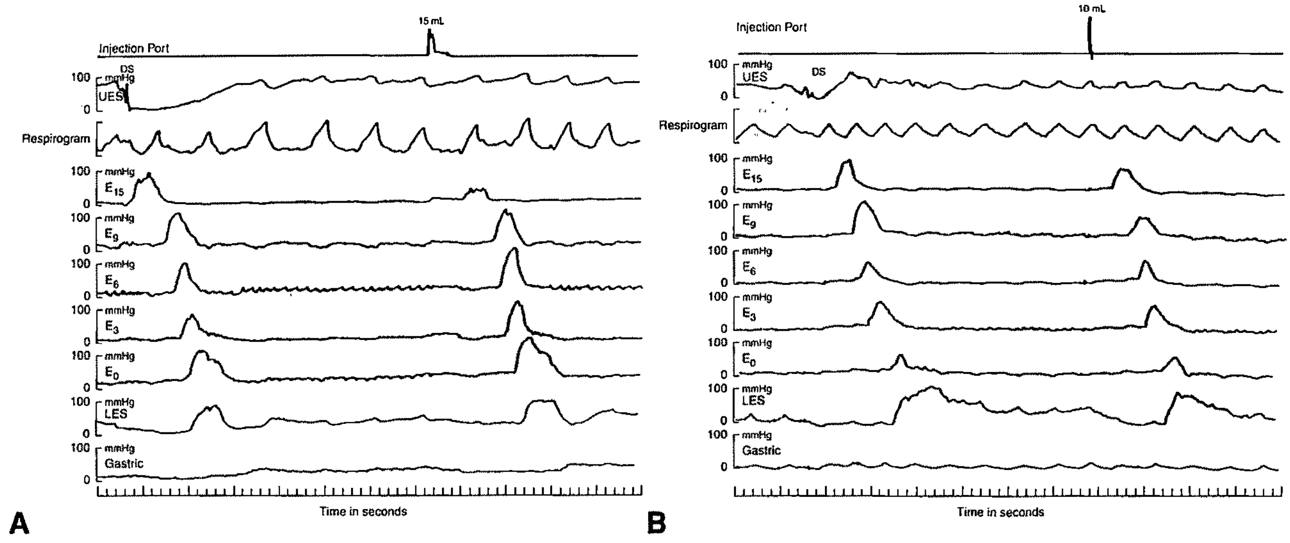


Fig 2. Representative examples of secondary esophageal peristalsis in A) control and B) posterior laryngitis patient. In both individuals, intraesophageal air injection of threshold volume (10 and 15 mL, respectively) resulted in progressive peristaltic wave contraction that started at 15 cm above LES (E15, etc) and propagated to stomach. These events were associated with complete LES relaxation. However, in these examples, intraesophageal air injection did not induce any change in UES pressure. In both examples, dry swallow (DS) and its associated primary peristalsis are shown before stimulation of secondary esophageal peristalsis. In contrast to secondary esophageal peristalsis, in these examples, primary peristalsis was associated with complete UES relaxation, as well as LES relaxation.

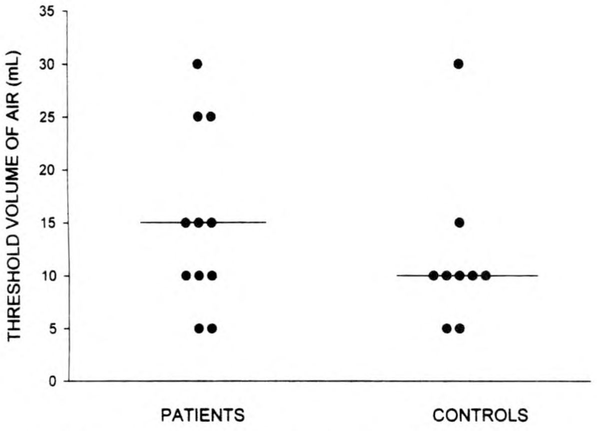


Fig 3. Comparison between controls and posterior laryngitis patients in terms of threshold volumes of intraesophageal injection of air required to stimulate secondary esophageal peristalsis. Threshold volume of each subject is depicted, and median values are shown with horizontal lines. Between-group comparison did not show any significant difference between threshold volume required for triggering secondary esophageal peristalsis in controls and that of patients with posterior laryngitis.

and no response in 39%. In the control group, the UES response to the injection of the threshold volume of air was contraction in 67% and no response in 33%.

The LES response to the injected threshold volume of air was complete relaxation (a nadir of the LES pressure drop that was within 3 mm Hg of the intragastric pressure) in 88% of the trials in both groups, and partial relaxation or no response in the rest.

DISCUSSION

In this study, we determined the similarity of the presence and integrity of stimulation of secondary esophageal peristalsis in patients with posterior laryngitis and age-matched controls. The study findings showed that not only is the threshold volume of air required for stimulation of secondary esophageal peristalsis in patients similar to that of age-matched controls, but the amplitude, duration, and velocity are also similar between the two groups. These findings eliminate abnormalities of secondary esophageal peristalsis as a contributory factor in the pathogenesis of reflux laryngitis. To determine whether the sequential step-up method used in this study may have obscured or affected the threshold volume for stimulation of secondary peristalsis, we studied 5 additional normal volunteers with both this technique and the random injection technique with volumes rang-

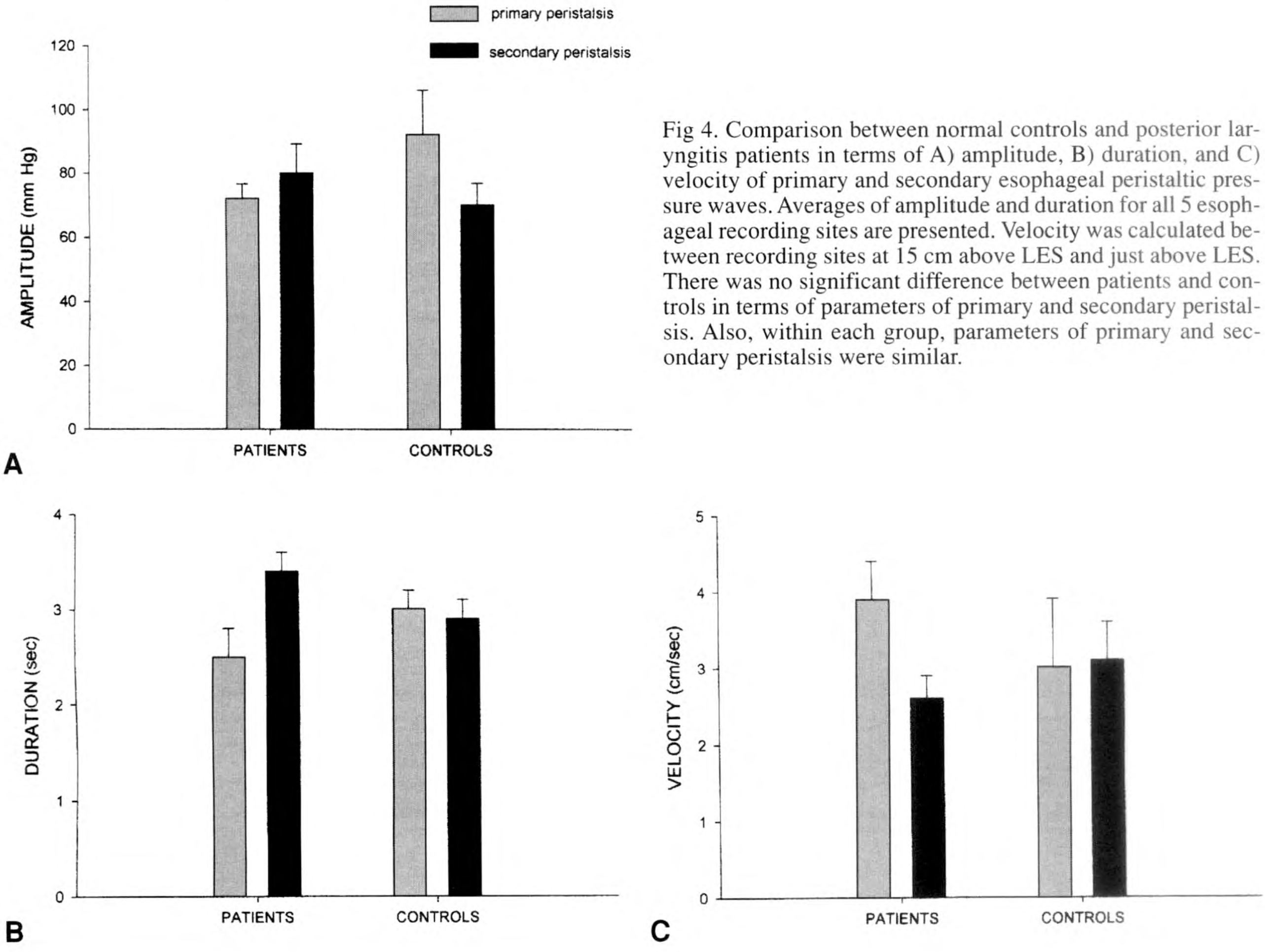


Fig 4. Comparison between normal controls and posterior laryngitis patients in terms of A) amplitude, B) duration, and C) velocity of primary and secondary esophageal peristaltic pressure waves. Averages of amplitude and duration for all 5 esophageal recording sites are presented. Velocity was calculated between recording sites at 15 cm above LES and just above LES. There was no significant difference between patients and controls in terms of parameters of primary and secondary peristalsis. Also, within each group, parameters of primary and secondary peristalsis were similar.

TABLE 2. COMPARISON OF STEP-UP TECHNIQUE VERSUS RANDOM VOLUME TECHNIQUE IN DETERMINING VOLUME OF AIR REQUIRED FOR STIMULATION OF SECONDARY PERISTALSIS

	Method	
Subject	*Randomized*	*Step-up*
1	15	15
2	5	5
3	10	10
4	5	5
5	5	5

ing from 5 to 60 mL. The results are shown in Table 2. The threshold volume for stimulation of secondary peristalsis was identical for both methods.

Protection of the aerodigestive tract structures from contact with gastric acid involves multiple mechanisms. These mechanisms can be divided generally into 2 groups: basal and response.[13,14] Basal mechanisms are those that are constantly maintained without a need for stimulation, although various stimuli may accentuate their function; basal mechanisms include the functions of the LES and UES. Response mechanisms are those that are not constantly active, but become activated upon stimulation. The stimulus for this group is generally distention of the esophagus and mechanical stimulation of the pharynx. This group includes secondary esophageal peristalsis, the esophago-UES contractile reflex,[15,16] the esophagoglottal closure reflex,[17,18] the pharyngo-UES contractile reflex,[19,20] the pharyngoglottal closure reflex,[21,22] and pharyngeal (secondary) swallow.[23,24]

The presence and integrity of these mechanisms in patients who have suffered the consequences of pharyngolaryngeal reflux of gastric acid, such as posterior laryngitis patients, have not been completely studied. One investigation[25] determined that the volume required for triggering the pharyngo-UES contractile reflex in this patient group is significantly higher than that of age-matched controls. The present study addresses another airway response mechanism protective against pharyngeal reflux of gastric acid. The findings of the present study, combined with those of the above-mentioned report,[25] indicate that abnormality of the airway protective mechanisms among patients with posterior laryngitis is not an all-or-none phenomenon, and that one or more of the mechanisms may function normally.

The similarity between the patient and control groups in terms of the parameters of the secondary and primary esophageal peristalsis reported in this study corroborates previous investigations that evaluated secondary peristalsis in healthy elderly subjects.[12]

The UES pressure response to generalized esophageal distention, such as that induced by air injection, is variable.[26] At a threshold volume, intraesophageal air injection induces complete relaxation and belching. Injections of volumes smaller than the threshold volume for stimulation of esophageal belching, in the majority of instances, result in contraction of the UES and, in the minority of instances, may induce partial relaxation or no effect. The findings of the present study in patients with posterior laryngitis are in concordance with the normal UES response to generalized air distention.

The LES response to generalized distention in complete or partial relaxation depends on the volume of injected air and the magnitude of the induced distention. Our findings of complete LES relaxation in an overwhelming majority of patients with posterior laryngitis and of partial relaxation in the remaining minority support preservation of a normal LES response to distention in this patient group.

In summary, secondary esophageal peristalsis is preserved in posterior laryngitis patients. The threshold volume of air required for triggering secondary esophageal peristalsis, and the parameters of secondary esophageal peristalsis such as amplitude, duration, and velocity, in posterior laryngitis patients are similar to those of age-matched normal controls. The UES and LES responses to esophageal distention by air in posterior laryngitis patients are similar to those in healthy controls.

REFERENCES

1. Meltzer SJ. On the causes of the orderly progress of the peristaltic movements in the esophagus. Am J Physiol 1899;2: 266-72.

2. Meltzer SJ. Secondary esophageal peristalsis of the esophagus. A demonstration on a dog with a permanent esophageal fistula. Proc Soc Exp Biol Med 1906;3:35-7.

3. Creamer B, Schlegel J. Motor responses of the esophagus to distention. J Appl Physiol 1957;10:498-504.

4. Patterson WG, Hynna-Liepert TT, Selucky M. Comparison of primary and secondary esophageal peristalsis in humans: effect of atropine. Am J Physiol 1991;260(Gastrointest Liver Physiol 23):G52-G57.

5. Siegel CI, Hendrix TR. Evidence for the central mediation of secondary peristalsis in the esophagus. Bull Johns Hopkins Hosp 1961;108:297-307.

6. Williams D, Thompson DG, Heggie L, Bancewicz J. Responses of the human esophagus to experimental intraluminal distension. Am J Physiol 1993;265(Gastrointest Liver Physiol 28):G196-G203.

7. Christensen J, Lund GF. Esophageal responses to distension and electrical stimulation. J Clin Invest 1969;48:408-19.

8. Paterson WG. Neuromuscular mechanisms of esophageal

responses at and proximal to a distending balloon. Am J Physiol 1991;260(Gastrointest Liver Physiol 23):G148-G155.

9. Ren J, Schulze-Delrieu K. Modulation of esophageal contractions by distension in vitro. Dig Dis Sci 1989;34:503-8.

10. Ren J, Schulze-Delrieu K. Movement of wax particles by contractions in the isolated opossum esophagus. Am J Physiol 1990;258(Gastrointest Liver Physiol 21):G164-G170.

11. Schoeman MN, Holloway RH. Integrity and characteristics of secondary oesophageal peristalsis in patients with gastroesophageal reflux disease. Gut 1995;36:499-504.

12. Ren J, Shaker R, Kusano M, et al. Effect of aging on the secondary esophageal peristalsis: presbyesophagus revisited. Am J Physiol 1995;268(Gastrointest Liver Physiol 31):G772-G779.

13. Shaker R. Airway protective mechanisms: current concepts. Dysphagia 1995;10:216-27.

14. Shaker R, Lang IM. Reflex mediated airway protective mechanisms against retrograde aspiration. Am J Med 1997;103 (suppl 3):64S-73S.

15. Torrico S, Kern M, Aslam M, et al. Upper esophageal sphincter function during gastroesophageal reflux events revisited. Am J Physiol 2000;279(Gastrointest Liver Physiol 42): G262-G267.

16. Reynolds RP, Effer GW, Bendel MP. The upper esophageal sphincter in the cat: the rôle of central innervation assessed by transient vagal blockade. Can J Physiol Pharmacol 1987;65:96-9.

17. Shaker R, Dodds WJ, Ren J, Hogan WJ, Arndorfer RC. Esophagoglottal closure reflex: a mechanism of airway protection. Gastroenterology 1992;102:857-61.

18. Shaker R, Ren R, Medda B, Lang I, Cowles V, Jaradeh S. Identification and characterization of the esophagoglottal closure reflex in a feline model. Am J Physiol 1994;266(Gastrointest Liver Physiol 29):G147-G153.

19. Shaker R, Ren J, Xie P, Lang IM, Bardan E, Sui Z. Characterization of the pharyngo-UES contractile reflex in humans. Am J Physiol 1997;273(Gastrointest Liver Physiol 36):G854-G858.

20. Medda BK, Lang IM, Layman R, Hogan WJ, Dodds WJ, Shaker R. Characterization and quantification of a pharyngo-UES contractile reflex in cats. Am J Physiol 1994;167(Gastrointest Liver Physiol 30):G972-G983.

21. Ren J, Shaker R, Dua K, Trifan A, Podvrsan B, Sui Z. Glottal adduction response to pharyngeal water stimulation: evidence for a pharyngoglottal closure reflex [Abstract]. Gastroenterology 1994;106:A558.

22. Shaker R, Medda BK, Ren J, Jaradeh S, Xie P, Lang IM. Pharyngoglottal closure reflex: identification and characterization in a feline model. Am J Physiol 1998;275(Gastrointest Liver Physiol 38):G521-G525.

23. Nishino T. Swallowing as a protective reflex for the upper respiratory tract. Anesthesiology 1993;79:558-601.

24. Shaker R, Ren J, Zamir Z, Sarna A, Liu J, Sui Z. Effect of aging, position, and temperature on the threshold volume triggering pharyngeal swallows. Gastroenterology 1994;107: 396-401.

25. Uluālp SO, Toohill RJ, Kern M, Shaker R. Pharyngo-UES contractile reflex in patients with posterior laryngitis. Laryngoscope 1998;108:1354-7.

26. Shaker R, Ren J, Podvrsan B, et al. Effect of aging and bolus variables on pharyngeal and upper esophageal sphincter motor function. Am J Physiol 1993;264(Gastrointest Liver Physiol 27):G427-G432.

Ann Otol Rhinol Laryngol 110:2001

THYROARYTENOID MUSCLE: FUNCTIONAL SUBUNITS BASED ON MORPHOLOGY AND MUSCLE FIBER TYPING IN CATS

RUI IMAMURA, MD
SÃO PAULO, BRAZIL

YOSHIKAZU YOSHIDA, MD
KURUME, JAPAN
TADASHI NAKASHIMA, MD
KURUME, JAPAN

HIROYUKI FUKUNAGA, MD
KURUME, JAPAN
MINORU HIRANO, MD
KURUME, JAPAN

Using parvalbumin immunohistochemistry to determine the distribution of muscle fiber types in the feline thyroarytenoid muscle (TA), we clearly distinguished the vocalis (with predominance of "slow" type 1 fibers) from the external TA (in which "fast" type 2 fibers predominated, especially in its rostral part). Reconstruction of serial frontal sections of the TA allowed the stereoscopic study of each division. The existence of a rudimentary laryngeal ventricle separating the true and false vocal folds in cats was demonstrated anatomically and histologically, and its relationships to each division of the TA were established. Our results suggest that the vocalis, fitted for enduring activities, is suited for voice control. The fast, rostral part of the external TA seems suited to laryngeal sphincteric demands, while its caudal counterpart may act in both functions. The anatomic individualization of the divisions of the TA may suggest that they play distinct physiological roles and may imply that they should not be considered a single functional unit.

KEY WORDS — animal structures, immunohistochemistry, laryngeal muscles, larynx, parvalbumin.

INTRODUCTION

The thyroarytenoid muscle (TA) is a complex intrinsic laryngeal muscle (ILM) that has been extensively studied in different mammalian species (for a review, see Suarez Nieto et al[1]). In some animals (dog, horse, and donkey), the TA is composed of 2 distinct muscles: the vocalis (VOC), located caudally, at the vocal fold level, and the ventricularis (VE), located rostrally, at the level of the vestibular fold.[2]

In the cat, although the TA is macroscopically undivided, histochemical studies have revealed regional differences in the composition of muscle fiber types[2] that allow the distinction of a mediocaudal portion (corresponding to the VOC) from the rest of the TA. Different physiological roles have been attributed to these portions,[2-4] but to date, an anatomic substrate for this statement is still lacking, since in many species, including the cat, the VOC has not been individualized along the whole length of the TA.

Muscle fiber typing of the ILMs of animals and human beings has been performed on the basis of reactions for myosin adenosine triphosphatase (ATPase) with or without histochemistry for oxidative or glycolytic enzymes.[2,4-11] Parvalbumin (PA) immunohistochemistry has been proposed as a sensitive technique for classifying muscle fibers into various subtypes.[12] Parvalbumin is a small, calcium-binding cytosolic protein that participates in the contraction-relaxation cycle of skeletal muscles. Its concentration is considered to influence the muscular relaxation speed, so that a higher content of PA is found in fast-contracting muscle fibers.[12-15] The PA immunostaining pattern has shown a good correlation with histochemical fiber typing by myofibrillar ATPase in skeletal muscles of rats.[12,14] In our previous studies in cats, we have shown that this method can be used to demonstrate the distribution of muscle fiber types in the ILMs[16] and pharyngeal muscles.[17]

The purpose of this study was to reveal the subdivisions of the cat's TA according to the distribution of muscle fiber types as determined by PA immunohistochemistry. Ultimately, by analyzing serial frontal sections of whole larynges, we intended to determine the 3-dimensional configuration of the divisions of the TA and describe the anatomic relationship between them and some of the laryngeal structures. To this end, gross anatomic and histologic studies were performed in order to confirm the existence of structures such as the laryngeal ventricle and vocal and vestibular folds in the cat's larynx, since controversy still exists regarding the presence[1,18,19] or absence[3,20,21] of such structures. We believe this knowledge may help us to better understand the different roles of this complex muscle in the cat's laryngeal physiology.

From the Department of Otorhinolaryngology–Head and Neck Surgery, Kurume University School of Medicine, Kurume, Japan.
CORRESPONDENCE — Rui Imamura, MD, Av. Liberdade, 91, cj. 91/93, São Paulo, SP, CEP 01503-000, Brazil.

TABLE 1. TISSUE PROCESSING AND STUDY DESTINATION

Animal	*Method*	*Plane of Sectioning*	*Study Destination*
Cats 1 to 8	NA	NA	Gross anatomy
Cat 9	Frozen section	Frontal	Histology
Cat 10	Frozen section	Frontal	PA (S/FT)
Cat 11	Frozen section	Frontal	Histology + PA (S/FT)
Kitten	Frozen section	Frontal	Histology + PA (S)
Cat 12	Paraffin embedding	Frontal	Histology
Cat 13	Paraffin embedding	Transversal	Histology + PA

NA — not applicable, PA — parvalbumin immunohistochemistry, S — serial reconstruction, FT — fiber typing.

MATERIALS AND METHODS

ANIMALS, LARYNGES, AND TISSUE PRETREATMENT

Larynges from 13 adult cats weighing from 2.0 to 2.3 kg and 1 kitten (0.6 kg) were used in this study. After deep anesthesia was induced by ketamine hydrocloride (25 to 35 mg/kg) and xylazine hydrochloride (0.3 to 0.6 mg/kg), the animals were perfused through the cannulated aorta with cooled Zamboni's fixative before removal of the larynx. All specimens were immersed in the same fixative and stored overnight at 4°C.

Eight larynges were destined for gross anatomic studies and did not undergo further treatment. The others, destined for histologic and/or immunohistochemical studies, were oriented for frontal (5 larynges) or transverse (1 larynx) sectioning. The larynx oriented for transverse sectioning and 1 of those oriented for frontal sectioning were embedded in paraffin, while all the others were frozen in liquid nitrogen before slicing (Table 1). Our preference for frontal plane sectioning relates to the convenience of this plane for analyzing the distribution of fiber types and the stereoscopic configuration of the TA in immunohistochemical studies. Sliding microtome serial sections (8 μm thick) from paraffin-embedded tissues and 14- to 20-μm-thick cryostat serial sections from frozen blocks were obtained and attached to poly-L-lysine–coated slide glasses. Before further processing, paraffin-embedded material was dewaxed and rehydrated.

GROSS ANATOMY

The larynges were sectioned in the midsagittal plane, and each hemilarynx was examined under an operative microscope according to the presence and location of the laryngeal ventricle and the vocal and vestibular folds. In 5 of the 8 larynges, the specimens were stained with 0.0005% toluidine blue ("Certistain" toluidine blue O; zinc chloride double salt; Merck, Darmstadt, Germany) in order to enhance the visual contrast of the different structures. The staining and washing times were 1 minute and 2 to 3 minutes, respectively. The mucosa of the right hemilarynx in each specimen was then carefully dissected for exposure of the TA, and the anatomic relationship of each division of this muscle to the aforementioned laryngeal structures was evaluated.

HISTOLOGY

The general architecture of the region surrounding the feline TA was studied by hematoxylin and eosin (H & E) and elastica–van Gieson (EVG) stains in the frontal and transverse planes. Sections were examined under light microscopy with special attention to the organization of elastic and collagenous fibers in the lamina propria of the laryngeal mucosa, the presence and location of any indentation that could correspond to the laryngeal ventricle, and the relationship of each division of the TA to neighboring structures, including their attachments to the laryngeal framework. Sections stained against PA antiserum were referenced in order to identify the limits of each division of the TA in corresponding sections stained with H & E or EVG. For this reason, the transversely sectioned larynx was also stained for PA immunohistochemistry, although it was not used for muscle fiber typing or serial reconstructions.

PARVALBUMIN IMMUNOHISTOCHEMISTRY

In both types of tissue pretreatment, the immunohistochemical protocol was identical. A detailed description of the immunohistochemical procedure is beyond the scope of this report and has already been given elsewhere.[22,23] Briefly, sections were incubated for 1 hour with 0.3% hydrogen peroxide and 10% normal horse serum in turn, in order to block endogenous peroxidase and nonspecific antibody sites. Subsequently, the sections were incubated with primary antibody (monoclonal anti-PA, mouse IgG1 isotype, clone PA-235, Sigma, St Louis, Mo), 1:2,000 dilution, at 4°C for 2 days; secondary biotinylated horse anti-mouse IgG (Vectastain Elite-ABC kit, Vector Labs, Burlingame, Calif), 1:200 dilution, at 4°C for 24 hours; and ABC complex (Vectastain ABC reagent, same kit), 1:100 dilution, for 1 hour. The peroxidase reaction was visualized with 3,3'-diaminobenzidine (DAB; DAB-kit, Vector Labs). False-positive immunoreactions were ruled out by incubating some sections without the primary antibody. No staining was observed in these sections.

Muscle Fiber Typing. A sample of PA immunore-

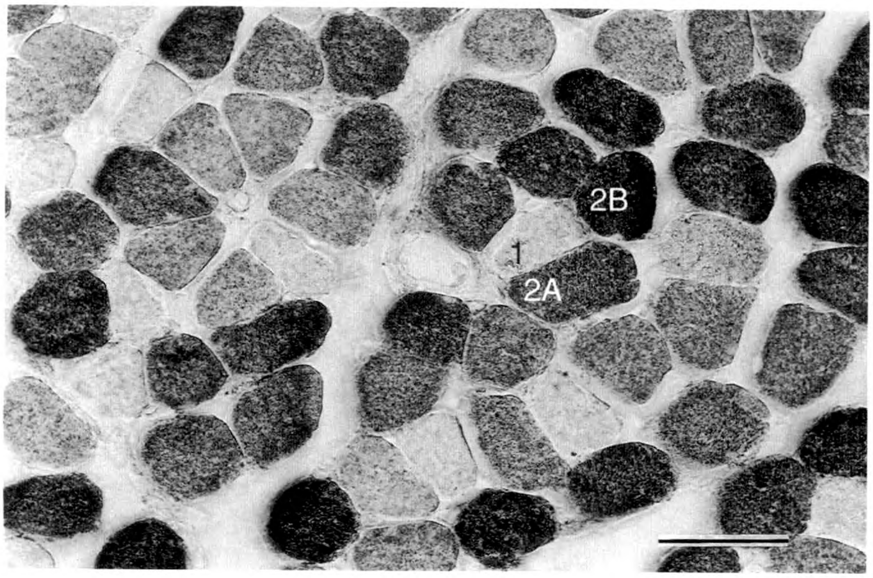


Fig 1. Example of parvalbumin (PA) immunohistochemistry in thyroarytenoid muscle (TA). Immunoreactivity of different types of muscle fibers (indicated in Figure) to PA antiserum is shown. According to Celio and Heizmann's[12] classification, most-stained fibers correspond to type 2B, while intermediate-stained ones correspond to type 2A. Type 1 fibers correspond to those that remained unreactive. Bar — 50 µm.

activity in a frontal section of the TA is shown in Fig 1.[12] Classification of muscle fibers in different subtypes was carried out according to the criteria described by Celio and Heizmann.[12] Regional differences in the composition of muscle fiber types in the VOC and external division of the TA (EXT) were investigated by typing all muscle fibers in a frontal section corresponding to the mid-portion of the muscle. In each division, different areas were arbitrarily demarcated, and the distributions (expressed as percentage of total fiber population) of each fiber type in these areas were determined and compared. In order to establish whether the distribution of fiber types in the VOC varied along its longitudinal axis, 2 other frontal sections, corresponding to its anterior and posterior segments, were selected for fiber typing. The distributions of fiber types in the rostral and caudal halves of the VOC in its anterior, middle, and posterior segments were then determined and compared.

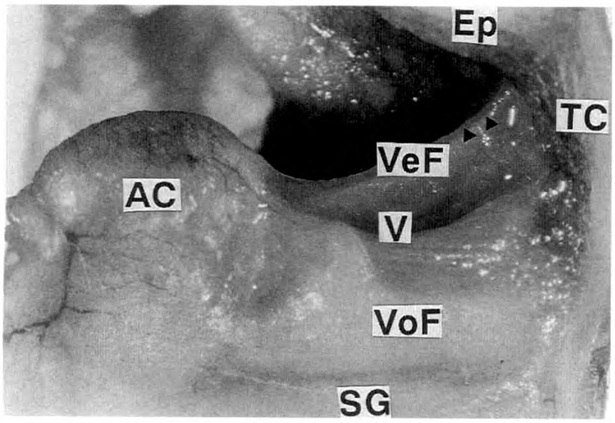


Fig 2. Gross anatomic specimen showing medial aspect of feline larynx. Small notch, corresponding to rudimentary laryngeal ventricle (V), separates vestibular folds (VeF) and vocal folds (VoF). Ep — epiglottis, TC — thyroid cartilage, AC — arytenoid cartilage, SG — subglottis, arrowheads — ventricular ligament.

Serial Reconstruction. Serial reconstruction of the VOC in relation to the EXT was carried out by mapping the limits of these divisions (defined by the composition of fiber types) in serial frontal sections of the larynx. Acquisition and digitization of data were performed with Neurolucida software (MicroBrightField, Inc, Colchester, Vermont) on a personal computer connected to a light microscope equipped with a drawing tube. All measurements, area calculations, and image generations were done with the same software. Although true 3-dimensional images could not be generated, the reconstructed structure had stereoscopic features, and could be rotated and viewed from any direction.

RESULTS

GROSS ANATOMY

In all cats investigated, a small notch was observed dividing the bulge of the thyroarytenoid fold into cranial and caudal portions. This notch was clearly seen extending through the whole length of the intermembranous glottis in 3 of the 8 specimens; in the others, it was detected only posteriorly, close to the arytenoid cartilage. We consider this notch to be a rudimentary laryngeal ventricle, separating the small vestibular fold, cranially, from the larger vocal fold, caudally (Fig 2). Toluidine blue stain enhanced the visual contrast between these structures. Careful examination of the vestibular fold with an operative microscope revealed, through the thin mucosa, a tenuous ventricular ligament extending from the base of the epiglottis to the apex of the arytenoid cartilage. After the mucosa was dissected, we observed that this ligament was topographically related to the rostral portion of the EXT.

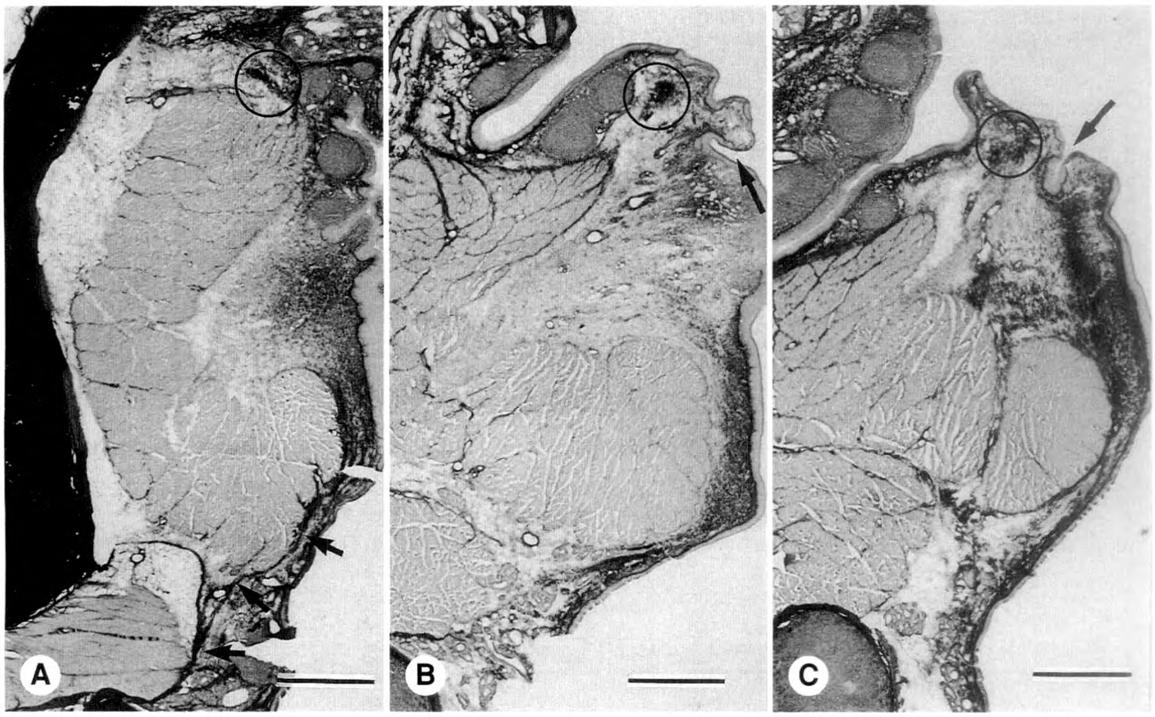


Fig 3. Frontal sections of A) anterior, B) middle, and C) posterior segments of larynx, stained with elastica–van Gieson method. Ventricular ligament (circles), consisting of elastic and collagenous fibers, can be observed in each segment just rostral to TA. Laryngeal ventricle (long arrows) can be identified in middle (B) and posterior (C) segments. Collagenous and elastic fibers are observed in thick lamina propria of mucosa just caudal to ventricle. They are concentrated in region medial to vocalis muscle in all segments (A-C), but extend toward cricoid cartilage as conus elasticus (short arrows) only in anterior portion of larynx (A). Bars — 1 mm.

HISTOLOGIC STUDIES

The histologic findings confirmed and complemented the observations made in the gross anatomic studies. The EVG stain revealed a rudimentary ventricular ligament, consisting of elastic and collagenous fibers, near the rostral extreme of the EXT (Fig 3). Serial frontal sections showed that it attached anteriorly to the thyroepiglottic ligament and posteriorly to the apex of the arytenoid cartilage. This ligament occurred systematically in all animals studied and was a landmark of the false vocal fold. Just caudal to it, there existed a small indentation, which was detected in the posterior half of the intermembranous glottis in all animals (Fig 3B,C). This indentation corresponded to the rudimentary laryngeal ventricle described in our gross anatomic specimens. Caudal to the laryngeal ventricle and rostral to the VOC, the mucosa of the vocal folds exhibited a thick lamina propria in which collagenous and elastic fibers were also observed. Nevertheless, they were not organized in a well-defined vocal ligament. These fibers were concentrated in the region just medial to the VOC and extended caudally to the cricoid cartilage, as did the conus elasticus. Different from that in humans, the elastic conus did not extend to the vocal process of the arytenoid cartilage and could be observed only in the anterior portion of the larynx (Fig 3A).

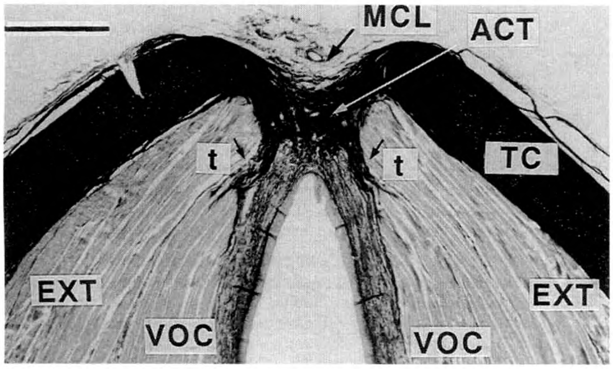


Fig 4. Transverse section of larynx at level of vocalis muscle (VOC) shows its anterior attachment (elastica–van Gieson method). MCL — median cricothyroid ligament, ACT — anterior commissure tendon, TC — thyroid cartilage, t — tendons from vocalis, EXT — external division of TA, bar — 1 mm.

In the anterior end of the VOC, at the level of the cranial portion of the medial cricothyroid ligament, many of the TA's fibers converged toward the anterior commissure tendon (ACT)[24] in a limited area between the laryngeal lumen and the thyroid cartilage. While muscle fibers in the EXT were seen reaching the ACT or the nearby thyroid cartilage's perichondrium, those of the VOC attached to the ACT, not directly, but through relatively long muscular tendons. These tendons represented approximately 10% of the

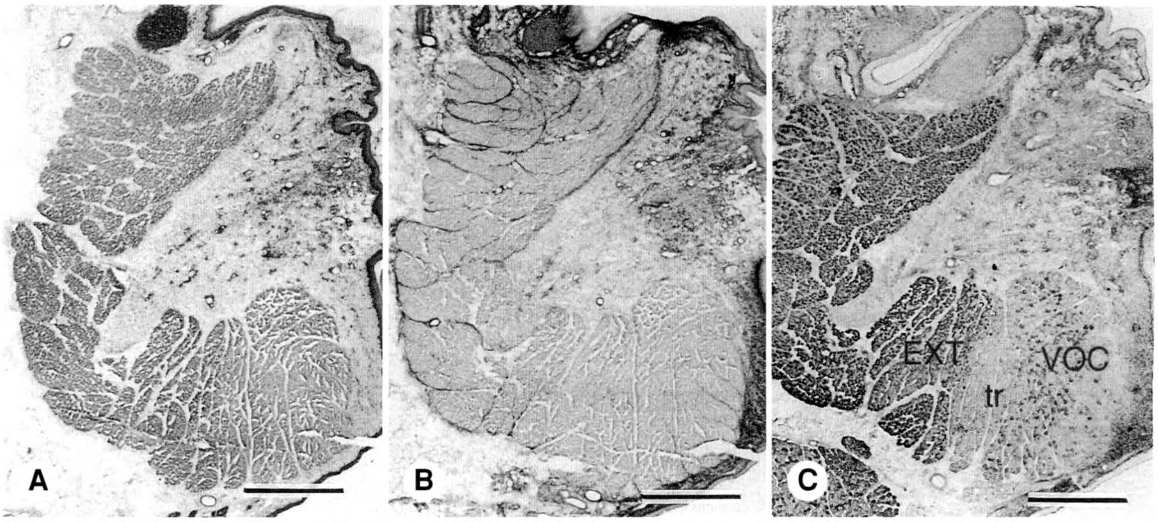


Fig 5. Histologic and immunohistochemical study of TA in frontal section. A) Hematoxylin and eosin. B) Elastica–van Gieson method. C) PA immunohistochemistry. Subdivisions of TA are only distinguished with PA. VOC — vocalis, EXT — external division of TA, tr — transitional area. Bars — 1 mm.

whole length of the VOC. As a consequence, there was a small segment in the anterior aspect of the TA constituted only by muscle fibers from the EXT (Fig 4).

In the posterior end, the muscle fibers of the VOC were seen reaching the lateroinferior border of the vocal process of the arytenoid cartilage, while those of the EXT attached to the anterolateral aspect of the body of the arytenoid.

THREE-DIMENSIONAL STRUCTURE OF TA

While H & E and EVG stains revealed the feline TA as a single muscle, PA immunohistochemistry allowed us to recognize an internal division and an external division in frontal sections of the TA. The former, corresponding to the VOC, exhibited a higher proportion of type 1 fibers and occupied the mediocaudal part of the muscle, while the latter, located in the rostrolateral part of the TA, revealed a predominance of type 2 fibers (Fig 5A). Although there was a small area of transition in the boundary between these two portions (Fig 5C), it was possible to distinguish the muscle fibers that belonged to the VOC from those of the EXT in its whole length. Transverse sections of the larynx reacted with PA antiserum revealed that this area of transition, rich in type 1 fibers, actually belonged to the EXT. Its posterior attachment was found in the anterior surface of the arytenoid body, together with the fibers of the most medial part of the EXT (Fig 6).

Serial reconstruction of the VOC and EXT allowed the analysis of the configuration, anatomic relationships, and morphometry of each division. No significant differences in the general structure of these di-

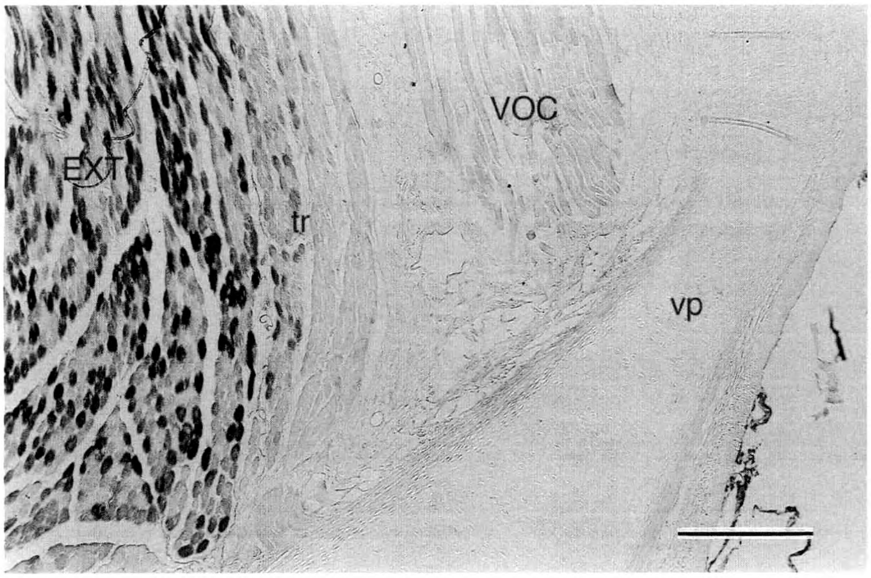


Fig 6. Transverse section of larynx shows posterior attachment of muscle fibers from vocalis (VOC), external division of TA (EXT), and transitional area (tr), stained for PA immunohistochemistry. Different attachment sites suggest that transitional area is not related to vocalis, even though it presents composition of muscle fiber types similar to that of vocalis. vp — vocal process of arytenoid cartilage, bar — 200 μm.

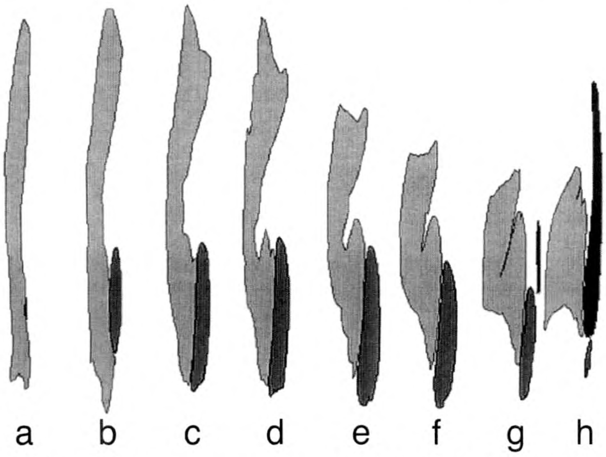


Fig 7. Serial reconstruction of vocalis (dark gray) and external division of TA (light gray). Arytenoid cartilage is represented in black in sections g (vocal process) and h (body). Sections a to h progress in anterior-to-posterior direction. For easier graphic illustration, only some sections are included. All sections are rotated 80° in vertical axis for 3-dimensional perspective. In consequence, mediolateral (transverse) dimension of each section is compressed. Real area occupied by each division of TA in frontal plane is shown in Table 2.

visions were observed among the animals studied.

Configuration. The EXT was relatively fan-shaped, with a large rostrocaudal extension anteriorly that decreased gradually toward the posterior part. Its configuration in the frontal plane corresponded to a straight column in the anterior part that gradually bent (with concavity toward the laryngeal lumen) as it progressed to the posterior end, in which it became polygonal. The structure of the VOC was much simpler; it corresponded to an ellipse in the frontal plane, so that it was ellipsoid on serial reconstructions (Figs 5C and 7).

Anatomic Relationship. The location of the VOC in relation to the EXT in the frontal plane varied according to the site along its longitudinal axis. In anterior segments, the VOC was eccentrically located, close to the caudal extreme of the TA, in its medial aspect (a and b on Fig 7). In the mid-portion of the muscle, as the EXT gradually bent, the VOC approximated the geometric center of the EXT (c through f on Fig 7). From this segment dorsally, toward the vocal process of the arytenoid, the lowermost portion of the VOC was on a vertical level inferior to or at least equal to that of the EXT. Finally, in posterior segments, the VOC tended to move away from the EXT and locate itself in the lateroinferior border of the vocal process of the arytenoid (g and h on Fig 7).

Relative Areas. Table 2 depicts the respective areas occupied by each division of the TA in the sections represented in Fig 7. In general, the area occupied by each division was greater in the middle portion and smaller at both ends of the muscle. The area of the EXT was much larger than that of the VOC, reflecting a larger number of muscle fibers. As it progressed posteriorly, however, the area of the VOC increased proportionally more than that of the EXT, and the ratio of the areas decreased, reaching a minimum of 2.8 close to the middle portion of the TA. The greatest area of the VOC occurred in a section slightly posterior to the mid-portion of the intermembranous glottis (e on Fig 7).

DISTRIBUTION OF FIBER TYPES

The 3 main fiber types (1, 2A, 2B) were identified in both divisions of the TA in the classic checkerboard distribution. The distributions of fiber types in the rostral and caudal halves of the anterior, intermediate, and posterior segments of the VOC were compared, but no significant difference was observed (Table 3). In all areas, type 1 fibers predominated, representing about 50% of all muscle fibers, while 2B fibers occurred in low percentages (around 5%). Contrasting with the relatively homogeneous distribution of fiber types in the VOC, regional differences in fiber type composition were observed in the EXT. The percentage of type 1 fibers was low in the rostral portion and gradually increased caudally, being greatest at the area of transition to the VOC (Fig 8 and Table 4).

DISCUSSION

In the present study, we investigated the distribution of PA immunoreactivity in the feline TA and adopted the criteria described by Celio and Heizmann[12] to classify muscle fibers as types 1, 2A, and 2B. Although we could not recognize type 2C fibers, they would not have interfered significantly in our results, since the occurrence of this fiber type in the ILMs of adult cats has been reported to be extremely low.[4]

The importance of and interest in determining the

TABLE 2. AREA OCCUPIED BY EACH DIVISION OF TA IN FRONTAL PLANE

Division of TA	*Section a*	*Section b*	*Section c*	*Section d*	*Section e*	*Section f*	*Section g*	*Section h*
Area of EXT (μm^2)	4,916,778	6,293,886	6,656,035	6,561,630	5,598,317	5,020,179	4,465,496	3,868,886
Area of VOC (μm^2)	20,550	729,529	1,523,670	1,774,716	1,990,084	1,803,614	717,137	102,164
Ratio EXT/VOC	239.2593	8.627333	4.368422	3.697285	2.813106	2.783401	6.22684	37.8694

Sections a to h refer to those represented in Fig 7.

TA — thyroarytenoid muscle, EXT — external division, VOC — vocalis muscle.

TABLE 3. DISTRIBUTION OF FIBER TYPES IN VOCALIS MUSCLE

		Section b		*Section e*		*Section g*		*Total*	
Region	*Fiber Type*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Rostral	1	235	51.6	460	48.9	129	48.9	824	49.6
	2A	201	44.2	438	46.5	121	45.8	760	45.8
	2B	19	4.2	43	4.6	14	5.3	76	4.6
Caudal	1	219	48.3	425	47.3	132	50.0	776	48.0
	2A	209	46.2	417	46.4	124	47.0	750	46.4
	2B	25	5.5	57	6.3	8	3.0	90	5.6
Total	1	454	50.0	885	48.1	261	49.5	1,600	48.8
	2A	410	45.2	855	46.5	245	46.5	1,510	46.1
	2B	44	4.8	100	5.4	22	4.0	166	5.1

Sections b, e, and g refer to those represented in Fig 7 and correspond, respectively, to anterior, intermediate, and posterior segments of vocalis muscle.

distribution of fiber types in skeletal muscles is related to the possibility of estimating the physiological properties of these muscles by knowing their fiber type composition. In a combined physiological-histochemical study in the cat, Burke et al[25] showed that type 1 fibers corresponded to slow-contracting, fatigue-resistant fibers, while type 2 were fast-contracting; the subgroups A and B of type 2 fibers corresponded to fatigue-resistant and fatigable fibers, respectively. While resistance to fatigue has been correlated to the oxidative enzyme capacity of muscle fibers, speed of contraction seems to better correlate with their myosin ATPase activity.[25,26]

Physiological studies on the muscular contractile properties in many animals have shown that the TA is a very fast muscle.[4,27-29] Accordingly, histochemical analysis of the TA in different animal species revealed low percentages (9% to 26%) of type 1 fibers,[4,5,8,10] indicating it is a fast-twitch muscle. Although considerable individual and interspecies variation in the proportion of type 1 and type 2 fibers has been reported in ILMs, including the TA,[5,6,11] it is also important to consider which portion of the TA is being evaluated in both physiological and histochemical studies — a detail that is often ignored. In various animal species, a great difference in the distribution of fiber types, and hence in corresponding contraction properties, will be expected if the specimen taken for analysis comes from the mediocaudal (VOC) versus the rostrolateral portion of the muscle.[2,4,8,12] In some cases, this difference was accentuated enough to allow the histochemical identification of subdivisions of the TA, even if, macroscopically, it constituted a single muscle.[2]

In the cat, Mascarello and Veggetti[2] and Yokoyama et al[4] reported that it was possible to distinguish 2 divisions of the TA by using reactions for myosin ATPase, associated or not with histochemistry for metabolic enzymes. According to these authors, the caudal or mediocaudal portion of the TA (which they referred to as the "pars vocalis") revealed a relatively high percentage of type 1 fibers when compared to the rest of the TA (referred to as the "pars ventricularis"), in which the percentage of type 2 fibers was very high.

Concerning the terminology of these subdivisions, we prefer "external division" (or EXT) instead of "pars ventricularis" to describe that portion of the

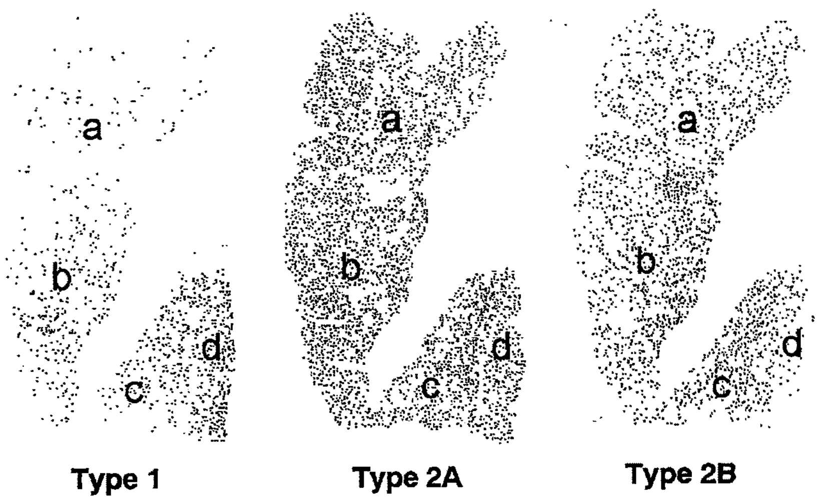


Fig 8. Distribution of muscle fiber types in frontal section of external division of TA in its midportion. Each dot represents 1 muscle fiber; fiber types 1, 2A, and 2B are shown separately for easier graphic illustration. Regions a to d represented in each illustration correspond to areas arbitrarily demarcated that were compared according to distribution of muscle fiber types. (Refer to Table 4.)

TABLE 4. DISTRIBUTION OF FIBER TYPES IN EXTERNAL DIVISION OF THYROARYTENOID MUSCLE

	Region a		*Region b*		*Region c*		*Region d*		*Total*	
Fiber Type	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
1	77	5.4	346	11.5	286	18.6	264	47.9	973	11.5
2A	846	59.2	1,629	54.0	747	48.7	248	45.0	3,470	54.0
2B	505	35.4	1,039	34.5	501	32.7	39	7.1	2,084	34.5

Regions a to d refer to those represented in Fig 8.

TA complementary to the VOC, since according to our histologic studies, only the rostral portion of the EXT was topographically related to the laryngeal ventricle and vestibular (or ventricular) fold. Therefore, we believe the term "ventricularis" should not be used to describe the whole EXT, but only its rostral portion. This was particularly evident in anterior segments of the muscle, in which the EXT had a large rostrocaudal extension and its inferior border was located at a lower vertical level than the VOC. Although regional differences in the distribution of fiber types in cross sections of the EXT have been observed[4] (as in this study), they were not accentuated enough to allow the recognition of further subdivisions of this muscle. Consequently, the caudal limits of the "pars ventricularis" remain indeterminate.

On the basis of anatomic and histologic studies, we showed that although very small, a rudimentary laryngeal ventricle could be consistently observed in the cat, cranial to the posterior half of the intermembranous glottis, separating the vocal and vestibular folds. This observation is in conflict with Negus'[20] classic description, which refers to the feline larynx as having an undivided thyroarytenoid fold and no ventricle, but coincides exactly with the report of Suarez Nieto et al.[1] A discussion of the relevance of such a small ventricle and such small vestibular folds in the physiology of the cat's larynx is beyond the purposes of this study. Nevertheless, we believe these structures constitute important anatomic landmarks and are pertinent from the point of view of comparative anatomy.

Our results on the configuration, fiber type distribution, and anatomic relationships to laryngeal landmarks suggest that the VOC, which parallels the vocal fold edge and presents a high percentage of type 1 fibers, may be able to perform fine tonus regulation of a given duration, being suited for voice control. The homogeneous distribution of fiber types in the VOC suggests that it represents a functional unit with no difference in physiological properties along its axis.

On the other hand, the EXT was heterogeneous in muscle fiber composition and revealed a more complex structure. Its ventricular (rostral) part exhibited a high proportion of fast-twitch fibers (in accordance with Mascarello and Veggetti[2]) and may be suited for protection of the airway at the supraglottic level. Although the relatively large epiglottis and the aryepiglottic folds provide some protection for the airway, during deglutition the feline larynx is ultimately sealed by contraction of the TA,[20] as confirmed by electromyographic studies of volitional swallow.[30] Furthermore, closure of the false vocal folds is particularly important for the cough reflex, since approximation of the true vocal folds alone cannot prevent egress of air from the lungs.[20] Although the valvular role of such small vestibular folds in cats can be questioned, a sphincteric action at this level may help in the rise of intrathoracic pressure necessary for coughing. Finally, the caudal portion of the EXT showed a composition of fiber types intermediate between that of the pars ventricularis and that of the VOC. This portion of the EXT may act together with the VOC to close the glottis[4] and may also influence the tension and volume of the vocal folds, particularly in the anteriormost portion of the glottis, in which muscle fibers of the VOC were absent.

An interesting aspect of Mascarello and Veggetti's[2] paper is that all ILM fibers of the species studied (dog, horse, donkey, cat, sheep, and ox) were positive for succinate dehydrogenase, with most fibers showing a very strong reaction for this oxidative enzyme. As oxidative activity is related to fatigue resistance, the authors concluded there were no fatigable (type 2B) fibers in the ILMs of these animals. On the other hand, if the results of myosin ATPase reaction at different pH levels in the same study[2] were considered alone, they would suggest the occurrence of type 2B fibers in the ILMs of the dog and the cat.

Similar histochemical inconsistencies have also been reported in skeletal muscles of rodents and were partly solved by the discovery of a previously unrecognized fiber type, 2X, in these animals.[31] The histochemical properties of type 2X fibers were in part similar to those of fiber type 2B and in part similar to those of type 2A, although they varied according to the animal species.[32] In the rat, 2X and 2B fibers revealed the same profile for myosin ATPase reaction at different pH preincubations, but the former showed an intermediate-to-dark reaction against oxidative enzymes. This finding is very similar to the one reported by Mascarello and Veggetti[2] in the cat's ILMs, and if confirmed in the cat, it would help to explain some of the inconsistencies reported by these authors. In the same manner, although some fibers were classified as 2B in both our study and that of Yokoyama et al[4] of the ILMs, the possibility exists that they correspond, in reality, to type 2X or another atypical fiber type. In fact, reports on skeletal limb muscles of rodents have correlated some of the fibers that had intermediate staining intensities for PA antiserum with type 2X fibers.[15] In the ILMs, the occurrence of atypical fiber types has recently been reported by means of histochemical, immunohistochemical, and electrophoretic techniques in the rat[33] and rabbit,[34] but a comprehensive study in different species, including the cat, is still lacking in the literature.

In humans, no difference in fiber type composition could be detected in the VOC and EXT.[2,6,10] However, particularities of ILM histochemistry, such as unusual or nonparallel staining for oxidative enzymes, especially of the TA and the posterior cricoarytenoid muscle, have been reported.[7,9,11] It is possible that some atypical or still-unrecognized muscle fiber type also occurs in the human ILMs, and if that is the case, the evaluation of its distribution may allow the individualization of subdivisions of the TA, as reported here in the cat.

Future studies on the larynx should consider this possibility and evaluate not only individual muscles, as defined macroscopically, but also individual parts of the same muscle that act differently from others, constituting a defined unit, suited to fill a specific demand on this specialized organ.

REFERENCES

1. Suarez Nieto R, Perez Casas A, Villa Vigil M. Morfologia comparada del musculo tiroaritenoideo. An Otorrinolarinol Ibero Am 1986;13:39-63.
2. Mascarello F, Veggetti A. A comparative histochemical study of intrinsic laryngeal muscles of ungulates and carnivores. Basic Appl Histochem 1979;23:103-26.
3. Pressman JJ, Kelemen G. Physiology of the larynx. Physiol Rev 1955;35:506-54.
4. Yokoyama T, Nonaka S, Mori S. Histochemical properties of intrinsic laryngeal muscles in cats. J Auton Nerv Syst 1995;56:50-60.
5. Sahgal V, Hast MH. Histochemistry of primate laryngeal muscles. Acta Otolaryngol (Stockh) 1974;78:277-81.
6. Teig E, Dahl HA, Thorkelsen H. Actomyosin ATPase activity of human laryngeal muscles. Acta Otolaryngol (Stockh) 1978;85:272-81.
7. Malmgren LT, Gacek RR. Histochemical characterization of muscle fiber types in the posterior cricoarytenoid muscle. Ann Otol Rhinol Laryngol 1981;90:423-9.
8. Ryu S. A histochemical study of intrinsic laryngeal muscle. Otologia (Fukuoka) 1982;28:394-402.
9. Lacau Saint Guily J, Fardeau M. Muscles intrinsèques du larynx de l'homme. Caractéristiques histoenzymologiques des fibres musculaires. Ann Otolaryngol Paris 1983;100:1-12.
10. Happak W, Zrunek M, Pechmann U, Streinzer W. Comparative histochemistry of human and sheep laryngeal muscles. Acta Otolaryngol (Stockh) 1989;107:283-8.
11. Claassen H, Werner JA. Fiber differentiation of the human laryngeal muscles using the inhibition reactivation myofibrillar ATPase technique. Anat Embryol (Berl) 1992;186:341-6.
12. Celio MR, Heizmann CW. Calcium-binding protein parvalbumin is associated with fast contracting muscle fibres. Nature 1982;297:504-6.
13. Heizmann CW, Berchtold MW, Rowlerson AM. Correlation of parvalbumin concentration with relaxation speed in mammalian muscle. Proc Natl Acad Sci U S A 1982;79:7243-7.
14. Heizmann CW, Celio MR. Immunolocalization of parvalbumin. Methods Enzymol 1987;139:552-70.
15. Füchtbauer EM, Rowlerson AM, Götz K, et al. Direct correlation of parvalbumin levels with myosin isoforms and succinate dehydrogenase activity on frozen sections of rodent muscle. J Histochem Cytochem 1991;39:355-61.
16. Fukunaga H, Imamura R, Yoshida Y, Nakashima T. Muscle fiber typing of the feline intrinsic laryngeal muscles showing immunoreactivity to parvalbumin. J Jpn Bronchoesophagol Soc 1998;49:350-6.
17. Imamura R, Yoshida Y, Fukunaga H, Nakashima T, Hirano M. Parvalbumin distribution in the musculature of the pharyngo-oesophageal segment. Acta Otolaryngol (Stockh) 1998; 118:875-81.
18. Fink BR, Frederickson EL, Gans C, Huggins SE. Evolution of laryngeal folding. Ann N Y Acad Sci 1976;280:650-7.
19. McClure RC, Dallman MJ, Garrett PD. Cat anatomy. An atlas, text and dissection guide. Philadelphia, Pa: Lea & Febiger, 1973.
20. Negus VE. The comparative anatomy and physiology of the larynx. New York, NY: Hafner, 1962.
21. Kelemen G. Comparative anatomy and performance of the vocal organ in vertebrates, and acoustic behaviour of animals. Amsterdam, the Netherlands: Elsevier, 1963.
22. Annoh H, Inokuchi T, Ohta K, Wakimoto M, Ueda T. Immunohistochemical investigations of parvalbumin localization in the skeletal muscle fibres of rats. Okajimas Folia Anat Jpn 1995;72:221-6.
23. Tanaka Y, Yoshida Y, Hirano M, Morimoto M, Kanaseki T. Distribution of SP- and CGRP-immunoreactivity in the cat's larynx. J Laryngol Otol 1993;107:522-6.
24. Broyles EN. The anterior commissure tendon. Ann Otol Rhinol Laryngol 1943;52:342-5.
25. Burke RE, Levine DN, Zajac FE, Tsairis P, Engel WK. Mammalian motor units: physiological-histochemical correlation in three types in cat gastrocnemius. Science 1971;174:709-12.
26. Barany M. ATPase activity of myosin correlated with speed of muscle shortening. J Gen Physiol 1967;50:197-216.
27. Hast MH. The primate larynx. A comparative physiological study of intrinsic muscles. Acta Otolaryngol (Stockh) 1969; 67:84-92.

28. Martensson A, Skoglund CR. Contraction properties of intrinsic laryngeal muscles. Acta Physiol Scand 1964;60:318-36.

29. Hall-Craggs ECB. The contraction times and enzyme activity of two rabbit laryngeal muscles. J Anat 1968;102:241-55.

30. Kobara-Mates M, Logemann JA, Larson C, Kahrilas PJ. Physiology of oropharyngeal swallow in the cat: a videofluoroscopic and electromyographic study. Am J Physiol 1995;268:232-41.

31. Schiaffino S, Gorza L, Sartore S, et al. Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. J Muscle Res Cell Motil 1989;10:197-205.

32. Gorza L. Identification of a novel type 2 fiber population in mammalian skeletal muscle by combined use of histochemical myosin ATPase and anti-myosin monoclonal antibodies. J Histochem Cytochem 1990;38:257-65.

33. DelGaudio JM, Sciote JJ, Carroll WR, Esclamado RM. Atypical myosin heavy chain in rat laryngeal muscle. Ann Otol Rhinol Laryngol 1995;104:237-45.

34. Lucas CA, Rughani A, Hoh JFY. Expression of extraocular myosin heavy chain in rabbit laryngeal muscle. J Muscle Res Cell Motil 1995;16:368-78.

≈

NINTH INTERNATIONAL FACIAL NERVE SYMPOSIUM

The Ninth International Facial Nerve Symposium will be held July 29 to August 1, 2001, in San Francisco, California. For information, contact Office of Continuing Medical Education, University of California, San Francisco; telephone: (415) 476-5808; fax: (415) 502-1795; e-mail: info@ocme.ucsf.edu.

Ann Otol Rhinol Laryngol 110:2001

RETROPHARYNGEAL AUTOLOGOUS FAT TRANSPLANTATION FOR CONGENITAL SHORT PALATE: A NASOMETRIC ASSESSMENT OF FUNCTIONAL RESULTS

PHILIPPE H. DEJONCKERE, MD, PHD HANS A. VAN WIJNGAARDEN, MD, PHD

UTRECHT, THE NETHERLANDS

Seventeen patients (4 to 24 years old; mean, 9.7 years) with mild velopharyngeal insufficiency were treated in our department during the period 1996 to 1999 with augmentation of the posterior pharyngeal wall with autologous fat. The main disorder was a congenital short palate without a cleft, in most cases revealed by adenoidectomy. Four patients had previously undergone pharyngoplasty, and 1 had already been injected in the posterior pharyngeal wall with Teflon paste. All patients had been exhaustively treated with speech therapy, and the result remained unsatisfactory. The functional outcome of the surgical procedure was quantified by acoustic nasometry. The decrease of the nasalance percentage for a standardized spoken passage was significant 1 to 3 months after the fat transplantation, and there was a slight tendency to further reduction of nasality at the late follow-up visit, more than 6 months (average, 9.4 months) after the intervention. The mean value of the nasalance score for the "normal passage" (running speech) then reached the limit of normal values. A long-term follow-up (average, 24.3 months) by telephone questionnaire confirmed the persistence of the beneficial results. Autologous fat seems an excellent alternative for Teflon in this indication. Acoustic nasometry allows a precise quantitative assessment of functional velopharyngeal surgery.

KEY WORDS — fat, nasalance, nasality, nasometry, pharyngoplasty, velopharyngeal insufficiency.

INTRODUCTION

Besides palatatoplasty techniques using a pharyngeal flap, augmentation pharyngoplasty, which aims to make the dorsal pharyngeal wall more bulky, is a classic surgical procedure for reducing hypernasal speech as a consequence of velopharyngeal incompetence.[1] However, augmentation pharyngoplasty is only recommended for mild cases of velopharyngeal insufficiency.[2] It can also be performed as a complementary procedure after pharyngoplasty with creation of a flap. Several kinds of material have been proposed as posterior wall implant: paraffin,[3] silicone,[4] homologous cartilage,[5] and, principally, Teflon (polytetrafluoroethylene).[6-8]

The literature — especially that concerning injections in vocal folds — mentions that in general, Teflon is well tolerated. However, the problem of development of Teflon granulomas (foreign body giant cell reaction) has been repeatedly emphasized. A particularity of these granulomas is that they seem to be unpredictable, and that they can occur a very long time (as many as 25 years) after the surgical procedure, even if the functional results (on voice) were satisfactory.[9]

Therefore, Mikaelian et al[10] and Brandenburg et al[11] proposed to replace Teflon with autologous fat for injection in vocal folds. This elicited the idea of also using fat transplantation for augmentation pharyngoplasty. It is interesting to note that as early as 1926, W. von Gaza[12] had already described such a procedure, albeit using an external cervical approach.

Seventeen patients (4 to 24 years of age; mean, 9.3 years) with a mild velopharyngeal insufficiency and hypernasal speech were treated in the period 1996 to 1999 with autologous fat transplantation in the posterior pharyngeal wall, and the functional results were objectively and subjectively investigated.

The functional assessment was specially focused on quantitative acoustic nasometry (Nasometer, Kay Elemetrics, Pine Brook, NJ); the reliability and validity of this technique have been recognized.[13-15] A nasometric measurement was performed before the surgical treatment, and 2 times (short-term and long-term) after surgery. Furthermore, still longer-term effects were checked by use of a questionnaire completed by a telephone inquiry.

MATERIAL AND METHODS

Patients, Diagnosis, and Criteria. Our 17 patients (11 male and 6 female) were mainly children and adolescents, ages 4 to 24 years (mean, 9.3 years). All of them had slight to moderate hypernasal speech — as perceptually rated by an experienced team comprising a phoniatrician and a speech therapist — and underwent videonasopharyngoscopy, X-ray contrast pharyngography, the classic aerodynamic tests with

---

From the Institute of Phoniatrics, Utrecht University, Utrecht, the Netherlands.

CORRESPONDENCE — Philippe H. Dejonckere, MD, PhD, Institute of Phoniatrics, Utrecht University, PO Box 85 500, 3508 GA Utrecht, the Netherlands.

a mirror, and nose auscultation during phonation tasks. In all cases, a mild velopharyngeal insufficiency was diagnosed, related to a constitutional disproportion between the length of the soft palate and the depth of the rhinopharynx. In 12 cases, the short palate was revealed after adenoidectomy. Five patients had previously undergone functional velopharyngeal surgery: 4 pharyngoplasties with a pharyngeal flap (in 1 case for a submucosal cleft) and 1 Teflon augmentation pharyngoplasty. Our series did not include patients with the velocardiofacial syndrome.

Our criteria for augmentation of the pharyngeal wall were 1) normal muscular activity with adequate lifting of the soft palate, 2) a distance between the velum and the dorsal pharyngeal wall of not more than 3 to 4 mm, as measured on the lateral pharyngogram, 3) absence of a severe language acquisition delay or mental impairment, and 4) lack of a significant effect of speech therapy.

Surgical Procedure. The procedure is always performed under general anesthesia with intubation. The fat is harvested from the periumbilical area via a small incision. The surrounding fascia and connective tissue is removed, leaving only fat globules, and the lipocytes are disturbed as little as possible. The fat globules are then loaded into a Brünings syringe. After lifting of the soft palate, the needle is transorally inserted just above the prominence caused by the tubercle of the atlas. The fat is injected submucosally with the Brünings syringe. The amount of injected fat tissue is variable: usually 2 to 3 mL in 1 medial site or 2 lateral sites, depending on the preoperative videonasopharyngoscopic observations and the outcome of X-ray contrast pharyngography. If a pharyngoplasty has been previously performed, the fat is positioned more caudally and laterally, in order to optimally narrow the nasopharyngeal ports.

Computer-Assisted Acoustic Nasometry. This technique consists of computing the nasal-oral acoustic ratio. During speech, the acoustic energy emanating from the nose and that emanating from the mouth are registered separately and properly filtered. The computer calculates the ratio of the acoustic energy emanating from the nose to the total acoustic energy (emanating from the nose and mouth together). This is expressed as a percentage defined by Fletcher[16] as "nasalance."

As in our clinical routine, we use 1) a standardized phonetically selected "normal" Dutch spoken passage and 2) a standardized phonetically selected "denasal" Dutch passage of comparable length. In the "normal" passage, the reading time is about 20 seconds. For young children, it can also be repeated sentence by sentence. The phonetic selection concerns the relative proportion of nasal and denasal speech sounds. In this "normal" passage, this ratio is similar to the general ratio in the Dutch language (in which 11.5% of the consonants are nasal). The average nasalance value in normal Dutch subjects is 31.95% (SD, 5.24%).[17]

The phonetic selection for the "denasal" passage concerns the proportion of nasal and denasal speech sounds. In this "denasal" passage, there are no nasal consonants. The average nasalance value in normal Dutch subjects is 11.75% (SD, 4.23%).[17] This "denasal" passage is supposed to be more sensitive for slight forms of hypernasality.

In a previous study, we found for the "normal" passage a correlation coefficient of .83 between the perceptual evaluation of nasality and the nasalance score, and for the "denasal" passage, a correlation coefficient of .77 between the perceptual evaluation and the nasalance score.[18]

In each case, a nasometric measurement was made before the intervention (after a trial treatment with speech therapy), then again between 1 and 3 months after operation (average, 2.2 months), and again, more than 6 months after operation (6 to 18 months; average, 9.4 months).

Telephone Questionnaire. In order to get more insight into the long-term results, we used a questionnaire completed by a telephone inquiry for the first 13 patients, at an average of 24.3 months (10 to 36 months) after the operation. In all cases except 1 (the only adult patient), the response was provided by the parents. The main (multiple choice) questions were "Have you experienced any change in speech since your last visit to our clinic?" (No change at all/slightly better/clearly better/slightly worse/clearly worse) and "Do you consider your speech now as normal or practically normal/slightly deviant/clearly deviant?"

RESULTS

Subjectively, all patients (or parents) declared they were satisfied. The speech of all patients was perceptually rated as less nasal after the operation. Neither local nor general complications were observed.

The objective quantitative scores are presented in Figs 1-3. Figure 1 shows the group values of the nasalance percentages (mean, standard error, and standard deviation) for the standardized "normal" and "denasal" passages. Figures 2 and 3 show the individual scores for the same conditions. Figure 2 presents the 13 patients for whom the fat transplantation was the primary surgical treatment, and Fig 3 presents the 4 patients who underwent the fat transplantation after a pharyngeal flap operation.

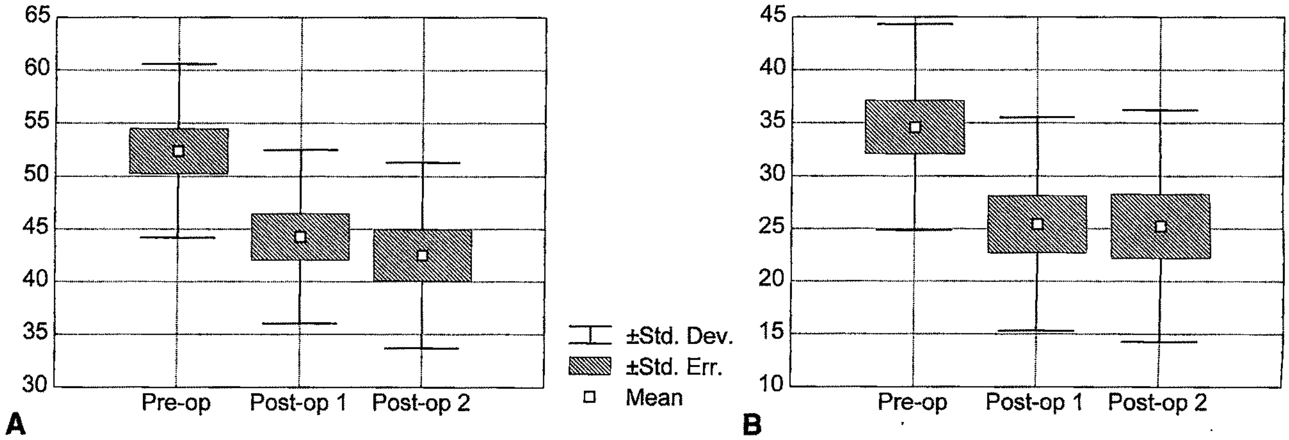


Fig 1. Nasalance percentages (mean value, standard error, and standard deviation) before fat injection, short time after fat injection, and long time after fat injection. A) Phonetically selected normal Dutch passage. Limit of normality is 42%. B) Phonetically selected denasal Dutch passage. Limit of normality is 22%.

In 4 cases, one of the postoperative measurements is lacking. In 1 case, the child could not be followed up within 3 months after surgery because of family circumstances, and in 2 cases the parents of the patient said they were satisfied after the short-term postoperative visit, and waived further examinations. One child was operated on recently.

A nonparametric Wilcoxon test for matched pairs was applied to check the significance of the observed changes. For the "normal" passage, comparison of the preoperative short-term group versus the postoperative short-term group showed a significant decrease of percent nasalance: p = .004 (n = 16). Comparison of the postoperative short-term group versus the postoperative long-term group showed a nonsignificant decrease of percent nasalance: p = .15 (n = 13). For the "denasal" passage, comparison of the preoperative short-term group versus the postoperative short-term group showed a significant decrease of percent nasalance: p = .002 (n = 16). Comparison of the postoperative short-term group versus the postoperative long-term group showed no significant change in percent nasalance: p = .5 (n = 13).

The responses to the 2 main questions of the questionnaire indicated that of the 13 patients, 5 had no perceptible change in speech since the last visit, 6 had a slight improvement of speech, and 2 had a slight worsening of speech; and that of the 13 patients, 6 had speech considered as normal or practically normal, and 7 had speech considered as still slightly deviant. No mention was made of any long-term adverse effect.

DISCUSSION

The reduction of nasopharyngeal air leakage by a rapid and technically simple injection of autologous fat into the posterior pharyngeal wall in order to build up a ridge appears to offer an interesting procedure for treatment of mild cases of velopharyngeal insufficiency or as an additional surgery after velopharyngoplasty.

The use of autologous fat would offer benefit not

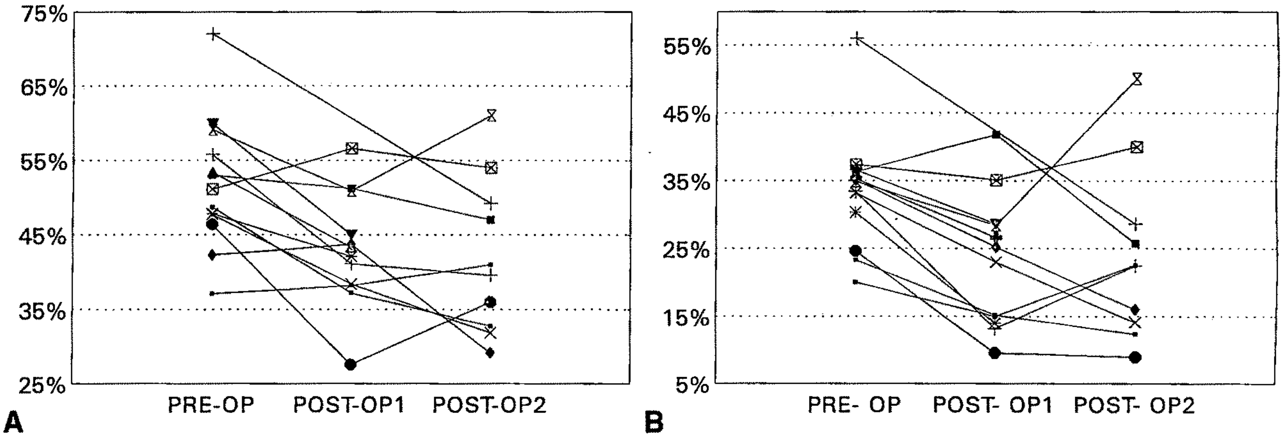


Fig 2. Individual nasalance scores per patient (13 cases with fat transplantation as primary treatment) before fat injection, short time after fat injection, and long time after fat injection. A) Phonetically selected normal Dutch passage. B) Phonetically selected denasal Dutch passage.

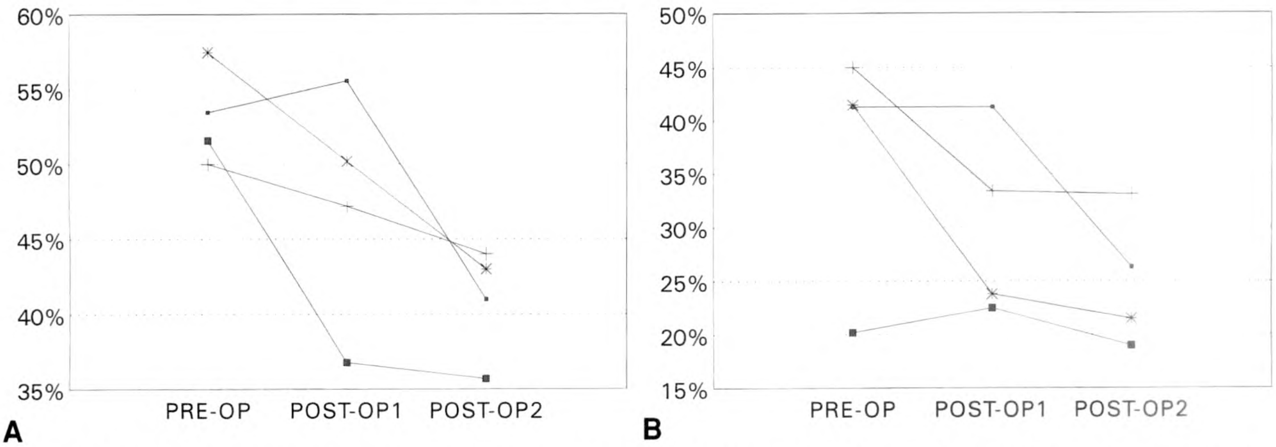


Fig 3. Individual nasalance scores per patient (4 cases with fat transplantation after pharyngoplasty) before fat injection, short time after fat injection, and long time after fat injection. A) Phonetically selected normal Dutch passage. B) Phonetically selected denasal Dutch passage.

provided by alloplastic materials,[11] because 1) the tissue is readily available and easily harvested and injected, 2) there is no risk of a foreign body reaction, and 3) there is no risk of a hypersensitivity reaction.

Because of its soft and pliable properties, fat tissue is very easy to implant, and the anatomic results are always very satisfactory. Figure 4 shows a sagittal magnetic resonance image taken 5 weeks after injection; the fat signal appears as a light area.

The ultimate fate of fat as an injectable implant continues to be debated, as there is always some extent of reabsorption. However, the long-term results with vocal fold injection are very encouraging; histologic studies have confirmed the preservation of viable fat at the injected site in dogs[19] and in human patients.[20,21] Fat can still be identified on magnetic resonance imaging scans more than 2 years after implantation, but it seems that microlipocysts and fibrous connective tissue are replacing damaged fat cells, which are gradually being reabsorbed.[22] We performed a histologic examination of fat tissue that appeared at the tip of the needle when it was pressed out of the Brünings syringe: it showed no perceptible morphological damage. Our late (>1 year) magnetic resonance imaging scans confirm the observations of Brandenburg et al[22]; although the bulging of the posterior pharyngeal wall remains, the fat signal becomes less obvious.

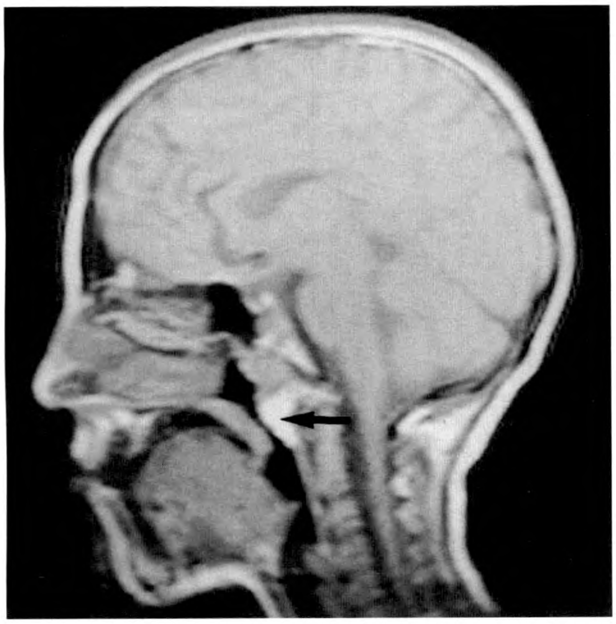


Fig 4. Magnetic resonance imaging scan performed 5 weeks after operation shows location (arrow) of injected adipose tissue in nasopharynx.

An interesting finding is that the reduction of hypernasality still lasts after the short-term effect. Improvement of the anatomic condition seems to provide new possibilities for further dynamic training. However, 1 of the patients showed, after an initially good result, a new increase of nasality between the short-term postoperative measure and the long-term postoperative measure. This finding is difficult to understand, as it did not correspond with the general clinical impression. A possible explanation is fatigue of the child at the moment of the nasometry.

CONCLUSION

In patients, especially children, who have mild velopharyngeal insufficiency due to a constitutional short palate (possibly revealed after adenoidectomy) and are resistant to speech therapy, transplantation of autologous fat in the dorsal pharyngeal wall to build up a ridge gives satisfactory functional results, as objectively quantified by acoustic nasometry. Further, unlike Teflon, autologous fat tissue will not cause granulomas. This procedure is also suitable for use after a previous pharyngoplasty with a flap.

In our study, there was a significant decrease in nasalance scores between the preoperative evaluation (after speech training) and an evaluation performed between 1 and 3 months (average, 2.2 months) after the operation. There was also a slight tendency to further reduction of the nasality at the late follow-up visit, more than 6 months (average, 9.4 months) after the intervention. The mean value of the nasalance score for a standardized "normal passage" (running speech) then reached the limits of a normal value. A long-term (average, 24.3 months) inquiry by telephone questionnaire confirmed the persistence of the beneficial results. No adverse effects were observed.

REFERENCES

1. Parton MJ, Jones AS. Hypernasality following adenoidectomy: a significant and avoidable complication. Clin Otolaryngol 1998;23:18-9.
2. Hirschberg J. Velopharyngeal insufficiency. Folia Phoniatr (Basel) 1986;38:221-76.
3. von Eckstein H. Hartparaffininjektionen in die hintere Rachenwand bei angeborenen und erworbenen Gaumendefekten. Klin Wochenschr (Berl) 1922;1:1185.
4. Brauer RO. Retropharyngeal implantation of silicone gel pillows for velopharyngeal incompetence. Plast Reconstr Surg 1963;51:254-8.
5. Trigos I, Ysunza A, Gonzalez A, del Carmen Vasquez M. Surgical treatment of borderline velopharyngeal insufficiency using homologous cartilage implantation with videonasopharyngoscopic monitoring. Cleft Palate J 1988;25:167-70.
6. Ward PH, Stoudt R, Goldman R. Improvement of velopharyngeal insufficiency by Teflon injection. Trans Am Acad Ophthalmol Otolaryngol 1967;71:923-33.
7. Bluestone CD, Musgrave RH, McWilliams BJ. Teflon injection pharyngoplasty — status 1968. Laryngoscope 1968;78: 558-64.
8. Smith JK, McCabe BF. Teflon® injection in the nasopharynx to improve velopharyngeal closure. Ann Otol Rhinol Laryngol 1977;86:559-63.
9. Varvares PA, Montgomery WW, Hillman RE. Teflon granuloma of the larynx: etiology, pathophysiology, and management. Ann Otol Rhinol Laryngol 1995;104:511-5.
10. Mikaelian DO, Lowry LD, Sataloff RT. Lipoinjection for unilateral vocal cord paralysis. Laryngoscope 1991;101:465-8.
11. Brandenburg JH, Kirkham W, Koschkee D. Vocal cord augmentation with autogenous fat. Laryngoscope 1992;102:495-500.
12. von Gaza W. Ueber freie Fettgeweibstransplantation in den retropharyngealen Raum bei Gaumenspalte. Arch Klin Chir 1926;142:590-9.
13. Haapanen ML. A simple clinical method of evaluating perceived hypernasality. Folia Phoniatr (Basel) 1991;43:122-32.
14. Stellzig A, Heppt W, Komposch G. Das Nasometer. Ein Instrument zur Objektivierung der Hyperrhinophonie bei LKG-Patienten. Fortschr Kieferorthop 1994;55:176-80.
15. Watterson T, Lewis KE, Deutsch C. Nasalance and nasality in low pressure and high pressure speech. Cleft Palate Craniofac J 1998;35:293-8.
16. Fletcher SG. Nasalance vs. listener judgements of nasality. Cleft Palate J 1976;13:31-44.
17. van de Weyer JC, Slis IH. Nasaliteitsmeting met de Nasometer. Logop Foniatr 1991;63:97-101.
18. Dejonckere PH, van Wijngaarden HA, Tsoukala M. Multifactorial approach of nasality assessment. In: Novak A, Last'ovka M, eds. Proceedings of the XVIII Congress of the Union of European Phoniatricians. Prague, Czech Republic: Empora Publishing, 1993:82.
19. Zaretsky LS, Shindo ML, deTar M, Rice DH. Autologous fat injection for vocal fold paralysis: long-term histologic evaluation. Ann Otol Rhinol Laryngol 1995;104:1-4.
20. Bauer CA, Valentino J, Hoffman HT. Long-term result of vocal cord augmentation with autogenous fat. Ann Otol Rhinol Laryngol 1995;104:871-4.
21. Shaw GY, Szewczyk MA, Searle J, Woodroof J. Autologous fat injection into the vocal folds: technical considerations and long-term follow-up. Laryngoscope 1997;107:177-86.
22. Brandenburg JH, Unger JM, Koschkee D. Vocal cord injection with autogenous fat: a long-term magnetic resonance imaging evaluation. Laryngoscope 1996;106:174-80.

Ann Otol Rhinol Laryngol 110:2001

COMPARISON OF THE MICROANATOMICAL DISTRIBUTIONS OF MACROPHAGES AND DENDRITIC CELLS IN NORMAL AND DISEASED TONSILS

JANET L. GORFIEN, MA
BERNICE NOBLE, PHD
LINDA BRODSKY, MD
BUFFALO, NEW YORK

The palatine tonsils play an important role in immunologic surveillance and resistance to infection in the upper aerodigestive tract. Dendritic cells and macrophages function to capture and process antigen and present it to T lymphocytes, a critical step in the early immune response. Few studies have characterized the distribution and phenotype of those antigen-presenting cells in the normal palatine tonsil, or determined how those parameters change with disease. Immunohistochemical analysis was performed to determine the microanatomical distribution, quantity, morphology, and phenotype of macrophages and dendritic cells in both normal and diseased tonsils. Differences were observed in macrophage and dendritic cell distribution, quantity, and phenotype in the surface and crypt epithelium. The number of macrophages was significantly increased in all compartments in all disease groups ($p < .05$), although the number of macrophages that expressed phenotypes of maturity and/or activation was not concomitantly increased. In the surface epithelium, Langerhans and interdigitating cells decreased significantly with disease ($p < .05$). Chronic infection may impose an immunosuppressive effect on responses within tonsil tissue, affecting the immunologic factors responsible for macrophage maturation and activation.

KEY WORDS — dendritic cell, immunohistochemistry, macrophage, tonsil.

INTRODUCTION

The palatine tonsils play an important role in immunologic surveillance and resistance to infection in the upper aerodigestive tract. Their unique structure allows both humoral and cell-mediated immune responses to develop upon appropriate antigen stimulation. Even in normal, healthy individuals, the tonsils can be in a highly reactive state, with both arms of the immune system actively functioning.[1]

Palatine tonsil tissue can be subdivided into several distinct, functionally interdependent microanatomical compartments: surface epithelium, crypt or reticulated epithelium, extrafollicular areas, and follicular areas.[1-6] Antigen-presenting cells, including macrophages and dendritic cells, may be found in variable numbers throughout the tonsil, and lie in close proximity to one another in subepithelial and extrafollicular compartments.[7] Dendritic cells and macrophages may collaborate to process and present antigen for T-cell activation.[8]

Recurrent tonsillitis and/or hyperplastic tonsils are commonly diagnosed in the pediatric population, although the cause of those disease entities is largely unknown. B cells and T cells increase in number in the tonsils of patients with recurrent tonsillitis and/or hypertrophy.[9,10] The number of dendritic cells also increases with disease.[11] Although macrophages and dendritic cells in the palatine tonsil have been studied in various disease states, those cells have not been well characterized in the normal pediatric population.[7,12-21]

This investigation was undertaken to evaluate the distribution of macrophages and dendritic cells in both normal and diseased tonsillar tissue in the pediatric population. A comparison of macrophage and dendritic cells in normal tonsils is essential for recognizing and understanding the potentially significant changes in location, number, and functional capacity that might occur in disease.

Previous studies have examined the functional state of macrophages and dendritic cells extracted from a variety of tissues and placed in tissue culture.[22,23] However, much information is lost when the microanatomical organization is disrupted. The main focus of the work reported here was to examine the location and phenotype of macrophages and dendritic cells within the context of the main microanatomical compartments of the tonsil. Changes in the functional status of macrophages and dendritic cells can be inferred from the phenotype of those cells, including the extent of major histocompatibility complex (MHC) class II expression. Other markers of activation include 2 monoclonal antibodies that distinguish macrophages from dendritic cells: RFD1 reacts with

---

From the Departments of Microbiology (Gorfien, Noble), Otolaryngology (Brodsky), and Pediatrics (Brodsky), State University of New York at Buffalo, School of Medicine and Biomedical Sciences, and the Children's Hospital of Buffalo, Buffalo, New York.

CORRESPONDENCE — Bernice Noble, PhD, State University of New York at Buffalo, Dept of Microbiology, 242 Cary Hall, 3435 Main St, Buffalo, NY 14214.

TABLE 1. MONOCLONAL AND POLYCLONAL ANTIBODIES

Antibody	*Dilution*	*Treatment*	*Source*
S-100	1:1,000	None	DAKO Corp, Carpinteria, Calif
MAC387	1:800	Trypsin	DAKO Corp
CD68	1:800	Trypsin	DAKO Corp
RFD7	1:80	None	Gift, Dr L Poulter, Royal Free Hospital, London, England
HLA-DR	1:400	Antigen retrieval	DAKO Corp
CD1a	Prediluted	None	Immunotech, Westbrook, Me
RFD1	1:80	None	Gift, Dr L. Poulter

mature interdigitating dendritic cells, and RFD7 with mature tissue macrophages.[24] RFD1 recognizes an HLA-DQ–associated antigen, and appears to be linked to antigen-presenting capabilities.[25,26]

The location, quantity, morphology, and phenotype of macrophage and dendritic cell subpopulations were analyzed by immunohistochemical methods. Differences among clinical and normal groups were evaluated. The relationship between germinal center size and the number of germinal center macrophages was also examined.

MATERIALS AND METHODS

TONSIL SPECIMENS

Tonsil tissue was taken from 39 children who underwent tonsillectomy for recurrent bouts of acute tonsillitis and/or obstructive tonsillar hyperplasia according to procedures described previously.[9,10] Three clinical classifications were established, based on both the estimated tonsil size and the presence of a clinical history of infection as previously described[9,10]: recurrent tonsillitis (RT; n = 12); idiopathic tonsillar hyperplasia (ITH; n = 15); and recurrent tonsillitis with persistent obstructive hyperplasia (RT/H; n = 12). Tonsil biopsy specimens of core tonsil tissue were obtained from 12 children who underwent surgery for noninfectious otolaryngological problems. The children had no previous history of ear, nose, and throat disease, and none had taken antibiotics for 6 months before surgery. On physical examination, their tonsils were small in size and normal in appearance. The study was approved by the Institutional Review Board of the Children's Hospital of Buffalo; participation required informed parental consent.

IMMUNOHISTOCHEMISTRY

Immediately after surgery, small pieces of tonsil tissue were placed in 10% neutral buffered formalin for either 24 hours (RT, RT/H, and ITH) or 4 hours (small control biopsy specimens). The tissues were subsequently routinely processed and embedded in paraffin.[27]

Tonsil tissue from each patient was also frozen in liquid nitrogen; sections were cut, air-dried, fixed in acetone, and stored at −20°C.

Paraffin sections were placed in xylene and rehydrated through a graded series of alcohol to distilled water. To achieve maximum epitope detection for CD68 and MAC387 monoclonal antibodies, proteolytic digestion was performed with a 0.1% trypsin/0.1% calcium chloride solution for 25 minutes at 37°C. An antigen retrieval technique was performed on tissue sections stained with the HLA-DR monoclonal antibody by means of a 10-mmol/L citrate buffer (pH 6.0) by the method of Tacha and Cham.[28] Endogenous peroxidase activity was suppressed with incubation in a 0.3% hydrogen peroxide solution for 20 minutes at room temperature. Six monoclonal antibodies (CD68, MAC387, HLA-DR, CD1a, RFD1, and RFD7) and 1 polyclonal antibody (S-100) were used to detect macrophages and dendritic cells in the tonsil tissue (Table 1). The staining characteristics and reactivity of those antibodies have been previously characterized and are summarized in Table 2.[13,15,24,29-37] Negative controls consisted of substi-

TABLE 2. CHARACTERISTICS AND REACTIVITY OF ANTIBODIES

Cell Type	*S-100*[13,36]	*CD68*[31-33]	*MAC387*[29,30]	*RFD7*[24]	*HLA-DR*[37]	*CD1a*[34,35]	*RFD1*[24]
Monocyte	−	+	+	−	+	−	−
Tissue macrophage	− (+)*	+	+	+†	±	−	−
Tingible body macrophage	−	+	−	−	+	−	−
Langerhans cell	+	−	−	−	+	+	− (+)*
Interdigitating dendritic cell	+	−	−	−	+	−	+
Indeterminate cell§	+	−	−	−	+	+	− (+)*
Squamous epithelium	−	−	+	−	−	−	−

+ — positive; − — negative; ± — weakly positive.

*Contrasting views in literature.

†Activated, mature tissue macrophages only.

§Indeterminate cells are distinguished from Langerhans cells by their lack of Birbeck granules.[13,15]

tuting nonimmune mouse IgG1 serum diluted to an equivalent concentration in place of the primary antibody. A Vector Elite immunoperoxidase staining kit (Vector, Burlingame, Calif) was used according to the manufacturer's instructions for the remainder of the staining procedure. A colored reaction product was developed with 3-diaminobenzidine tetrachloride (DAB; Sigma, St Louis, Mo).

DOUBLE STAINING

A sequential double staining technique was performed with certain combinations of antibodies: CD68 and HLA-DR, and CD68 and S-100. The details of this method have been previously published.[27] Briefly, the first antibody was stained as described above with the Vector Elite immunoperoxidase staining kit and with DAB used as the chromogen. An avidin-biotin blocking kit (Vector) was then applied to the tissue sections. The second antibody was detected with a Vector alkaline phosphatase staining kit. A colored reaction product was developed with a Vector alkaline phosphatase substrate kit. No counterstain was applied.

QUANTIFICATION OF STAINED CELLS

The tonsil specimens were evaluated in 5 microanatomically distinct compartments of the tonsil: the surface epithelium, crypt epithelium, germinal centers, extrafollicular areas, and blood vessel lumens. The sections were examined without knowledge about clinical classification. From 10 to 25 high-power fields (400×) were evaluated in each of the aforementioned compartments (4 to 25 high-power fields for control specimens) to determine the number of positive macrophages. The percentage of vessels with marginating monocytes, and the average number of monocytes per blood vessel were also calculated. Data are reported as the mean values of the individual means of observations for each patient in a clinical group.

In a previous study, germinal center size was determined with image analysis for each clinical disease category, as well as for control tonsils.[38] Data were reported as the mean germinal center areas for all samples in a clinical group, derived from the mean area for each individual. These results were compared to germinal center counts of macrophages, and a correlation value (r^2) was calculated.

STATISTICAL TESTS

Data were analyzed by correlation coefficients and Student's *t*-test for independent samples. A p value of .05 or less was judged significant.

RESULTS

The cells in all tonsil sections displayed crisp, intense cytoplasmic staining with all antibodies. The morphology of positive cells was consistent with that of macrophages and dendritic cells. The slides incubated with either nonimmune mouse serum or nonimmune rabbit serum were completely negative. The tissue sections stained with the monoclonal antibody MAC387 displayed very strong epithelial cell staining,[29,30] which precluded unambiguous identification of MAC387-positive macrophages in epithelia. For that reason, the counting of macrophages in the epithelial compartments was limited to CD68 staining.

NORMAL TONSIL CONTROLS

The epithelium of normal tonsils consists of pseudostratified squamous epithelium (on the surface) and reticulated epithelium (lining the crypts). Although both types of epithelium contained macrophages and dendritic cells (Langerhans cells, indeterminate cells, and interdigitating cells), differences were noted in the location and quantity of those cells (Table 3). Within the surface epithelium, dendritic cells (S-100 and RFD1) were most common (Fig 1A), with equal numbers of S-100– and RFD1-positive cells. Langerhans cells (CD1a; Fig 1C) were primarily located within the lower half of the epithelium. Macrophages (CD68) were few in number, and also stained with RFD7, a marker for mature macrophages (Fig 1E,G). The normal crypt epithelium contained significantly more macrophages (CD68) than the surface (Table 4 and Fig 1E,F). More RFD7-positive mature macrophages were present within the crypt than the surface epithelium (Fig 1G,H). In contrast, fewer Langerhans cells (CD1a) were present within the crypt epithelium than on the surface (Fig 1C,D). RFD1-positive dendritic cells, observed within the crypt epithelium, could not be reliably counted because of concomitant staining of B cells. Substantial numbers of S-100–positive cells were detected within the crypt epithelium, and were most likely to be interdigitating cells. S-100–positive cells were significantly more common within the crypt than the surface epithelium.

A well-described subset of the macrophage population, the tingible body macrophage, is found in germinal centers. Easily recognized by distinctive histomorphology, those cells can also be stained with the CD68 antibody, and are thought to play an important role in phagocytosis of apoptotic cells.[39] The positive cells were large and irregular, with a foamy cytoplasm containing distinct granules. The germinal centers of normal control tonsils contained large numbers of tingible body macrophages. MAC387 antibody revealed a subpopulation of smaller macrophages within germinal centers; those macrophages were few in number, as were S-100–positive cells.

In normal control tonsils, extrafollicular areas con-

TABLE 3. CELL COUNTS IN TONSIL TISSUE

Antibody	*Control (n = 12)*	*RT (n = 12)*	*RT/H (n = 12)*	*ITH (n = 15)*
Surface epithelium				
S-100	11.8 ± 3.1	13.3 ± 1.9	9.4 ± 0.9	11.2 ± 1.6
CD68	2.4 ± 0.7	5.5 ± 1.1[a]	6.5 ± 1.2[a]	4.2 ± 1.0
RFD7	2.6 ± 0.8	2.8 ± 0.8[b]	2.2 ± 1.2[c]	0.4 ± 0.1[a,d]
HLA-DR	7.4 ± 1.4	5.7 ± 1.0	5.6 ± 0.7	5.6 ± 0.5
CD1a	9.0 ± 0.8	3.1 ± 0.6[e]	4.0 ± 0.7[e]	4.2 ± 0.6[e]
RFD1	17.8 ± 4.0	6.5 ± 1.2[a,c]	6.5 ± 1.4[a]	5.4 ± 1.2[a,c]
Crypt epithelium				
S-100	22.1 ± 4.0	26.8 ± 2.6	30.3 ± 3.1	30.0 ± 4.0
CD68	15.7 ± 2.4	25.1 ± 2.4[a]	35.6 ± 3.8[e]	31.0 ± 2.9[a]
RFD7	5.2 ± 0.8[d]	7.9 ± 1.2[d]	6.0 ± 0.7[d]	6.5 ± 1.1[d]
CD1a	2.2 ± 0.5	0.5 ± 0.1[a]	0.4 ± 0.1[a]	0.6 ± 0.2[a]
Extrafollicular area				
S-100	20.6 ± 5.3	27.2 ± 2.8	24.1 ± 4.4	33.0 ± 6.5
MAC387	11.4 ± 2.3	18.1 ± 1.4[a]	21.9 ± 2.6[a]	15.2 ± 1.8
RFD7	14.0 ± 2.8	16.1 ± 3.1	9.7 ± 2.0	8.9 ± 2.0
Germinal center				
S-100	1.0 ± 0.3	0.6 ± 0.1	1.2 ± 0.4	1.6 ± 0.6
CD68	21.3 ± 2.8	41.5 ± 4.8[a]	43.0 ± 4.4[e]	54.4 ± 5.5[e]
MAC387	0.5 ± 0.2	1.7 ± 0.7	2.3 ± 0.5[a]	3.0 ± 1.3

Data are mean ± SE; count per high-power field.

RT — recurrent tonsillitis, RT/H — recurrent tonsillitis with persistent obstructive hyperplasia, ITH — idiopathic tonsillar hyperplasia.

[a] $p < .05$ compared with controls (see text).

[b] $p < .02$ compared with ITH (see text).

[c] $p < .04$ compared with CD68 cell count (see text).

[d] $p < .01$ compared with CD68 cell count (see text).

[e] $p < .001$ compared with controls (see text).

tained many MAC387- and RFD7-positive macrophages, as well as S-100–positive dendritic cells. No CD1a-positive Langerhans cells were detected within that compartment. RFD1-positive cells were observed within the extrafollicular area, but could not be reliably counted because of concomitant B cell staining.

The blood vessels within the normal control tonsils exhibited little evidence of monocyte margination. When margination was evident, only a few monocytes were detected.

COMPARISON OF NORMAL AND DISEASED TONSILS

Surface Epithelium. Table 3 provides a summary of the cell counts (per high-power field) for staining with all the antibodies in all tissue compartments. Table 3 also includes statistical assessments of the differences among patient groups and controls for each antibody in each tonsil tissue compartment.

S-100–positive cells were found in the surface epithelium in all groups studied, with no significant differences among clinical groups.

Expression of the activation or maturity marker RFD1 was reduced in patients and was also significantly lower than S-100 antigen expression (Fig 2).

The normal controls had more CD1a-positive Langerhans cells in the surface epithelium than any disease group (Figs 1C and 3A).

More CD68-positive macrophages were found in disease groups than in normals (Fig 3C).

In all cases, RFD7-positive cells were as frequent as in normal controls, but significantly fewer of those cells stained with CD68, a finding distinctly different from the normal condition, in which all CD68-positive cells were also RFD7-positive. Further, the ITH group had significantly fewer mature macrophages than the RT group (Fig 3E).

HLA-DR–positive cells were found in all tonsil tissues examined, with no significant difference among clinical groups.

Crypt Epithelium. S-100–positive cells were detected in the crypt epithelium, although there were no significant differences among clinical groups.

CD1a-positive Langerhans cells were reduced in patients as compared to controls (Figs 1D and 3B).

Macrophages stained with CD68 were more abundant in the crypt than the surface epithelium. Macrophages were more numerous in the crypt epithelium of diseased tonsils than in that of controls (Figs 1F

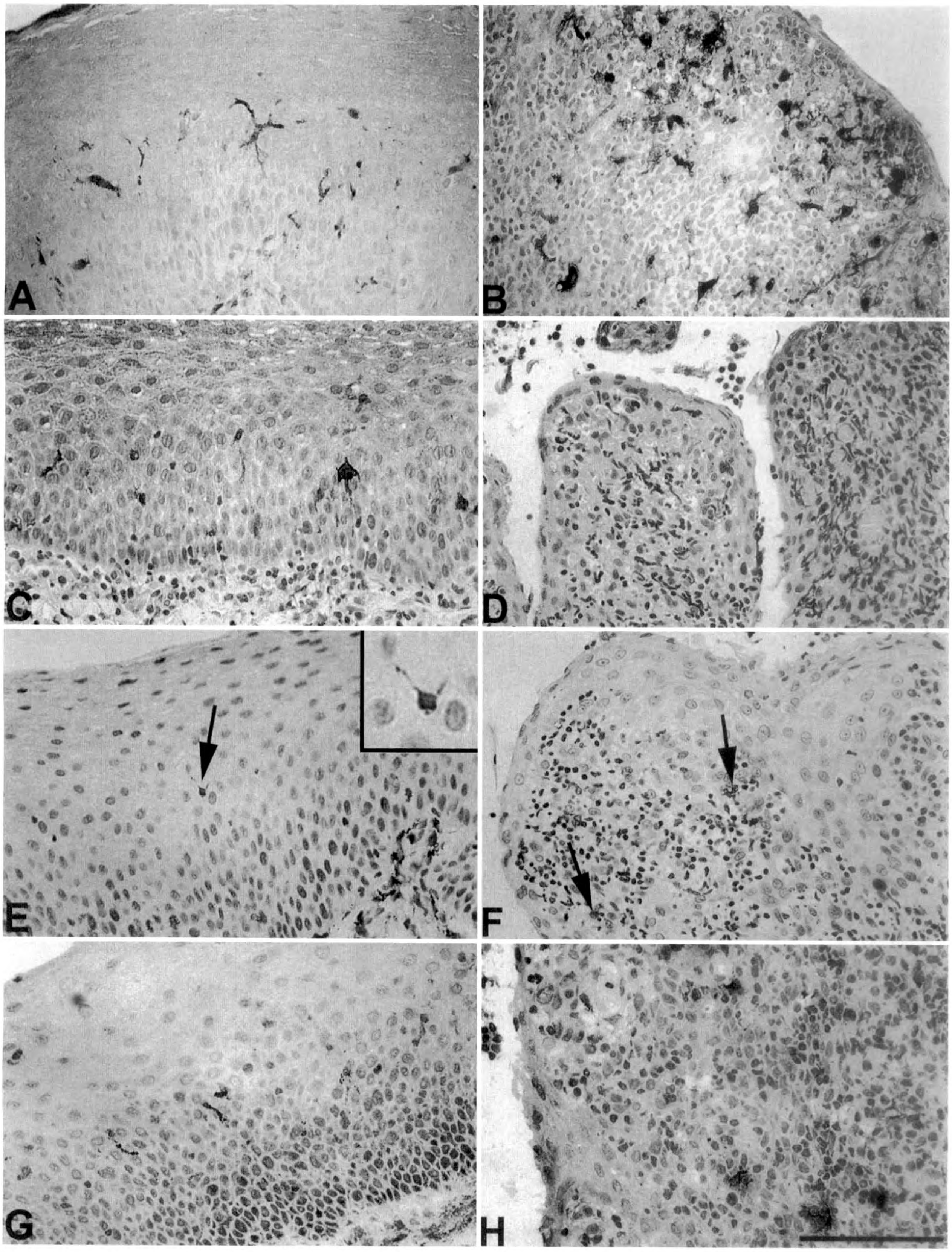


Fig 1. Surface (A,C,E,G) and crypt (B,D,F,H) epithelia of normal tonsils (original ×400). Bar — 100 µm. A,B) S-100 staining of dendritic cells. C,D) CD1a staining of Langerhans cells. E,F) CD68 staining of macrophages. Arrows — positive cells. Inset) Macrophage in surface epithelium. G,H) RFD7 staining of mature macrophages.

TABLE 4. COMPARISON OF SURFACE AND CRYPT EPITHELIA IN NORMAL TONSILS

Antibody/Cell Type	*Surface*	*Crypt*	*p*
CD1a/Langerhans cell	9.0 ± 0.8	2.2 ± 0.5	<.000001
CD68/macrophage	2.4 ± 0.7	15.7 ± 2.4	<.0001
RFD7/mature macrophage	2.6 ± 0.8	5.2 ± 0.8	<.05
S-100+ cell	11.8 ± 3.1	22.1 ± 4.0	<.05

and 3D).

Although RFD7-positive macrophages were equally frequent in all patient samples, they were less numerous than CD68-positive macrophages (Fig 3D,F). The high significance of the difference reflects the virtual absence in diseased tonsils of cells expressing the RFD7 phenotype, which is linked to macrophage maturity and activation.

Germinal Center. Tingible body CD68-positive macrophages were more numerous in patients than in controls.

S-100–positive cells were detected with equal frequency among all clinical groups.

In a previous study of germinal center areas determined by image analysis,[38] normal tonsils were found to have smaller germinal centers than tonsils from any of the disease groups. The tonsils from patients with ITH had the largest germinal centers. In this study, a positive correlation was noted between CD68-positive tingible body macrophages and germinal center areas ($r^2 = .367$; Fig 4).

Extrafollicular Area. The numbers of MAC387-positive macrophages were significantly elevated in the RT and RT/H groups, but the ITH group did not differ from the normal group.

Although RFD7-positive macrophages were found with equal frequency in all clinical groups, their numbers in the RT/H and ITH groups were decreased compared to MAC387 positivity. The discrepancy indicated that many of the macrophages were not mature, activated cells.

S-100–positive cells were found with equal frequency among all clinical groups.

Blood Vessel Lumens. The control samples had significantly fewer blood vessels that exhibited margination of monocytes than any diseased tonsils (Table 5). The ITH group had the highest percentage of blood vessels with margination.

A difference was also found in the average number of monocytes in those vessels with margination. The controls had a significantly lower number of monocytes within blood vessels that exhibited margination than any disease group (Table 4). Marginating monocytes were most numerous among the ITH group, with a strong correlation between the percent of vessels that exhibited margination and the number of monocytes in those vessels ($r^2 = .662$), indicating that monocytes were being recruited from the circulation.

Double Staining. Double staining for S-100– and CD68-positive cells revealed that macrophages and dendritic cells lie in close proximity to one another within the surface and crypt epithelia, confirming results reported by Okato et al.[7]

Double staining with HLA-DR and the macrophage marker CD68 revealed very little overlap in staining, showing that in general, CD68-positive macrophages in these tissues did not express MHC class II antigens, consistent with the view that these HLA-DR–positive cells are dendritic cells, most probably Langerhans cells.

DISCUSSION

It is necessary to determine the location, quantity, morphology, and activation state of immune cell populations within normal tonsil tissue in order to un-

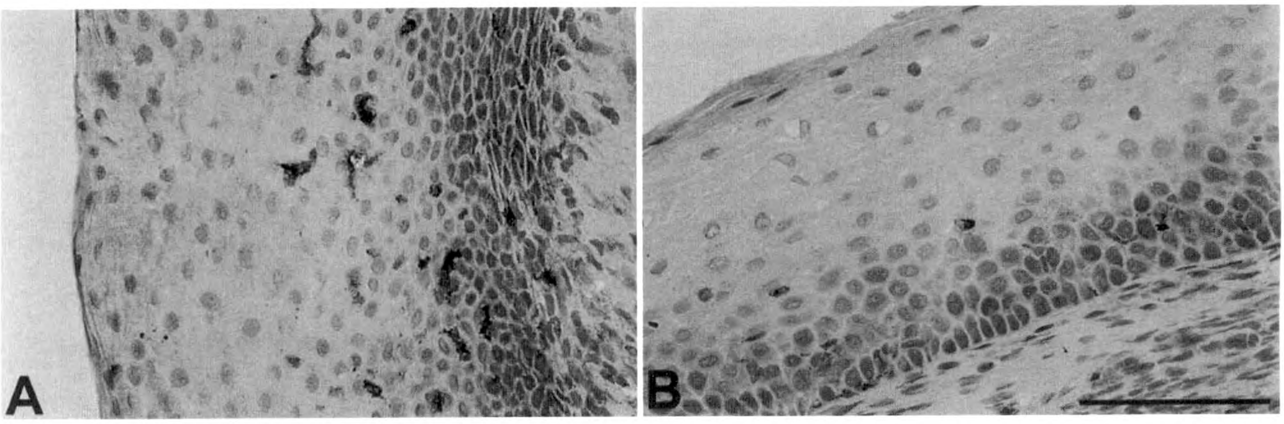


Fig 2. RFD1 staining of dendritic cells in surface epithelium of A) normal and B) diseased tonsils (original ×400). Bar — 100 µm.

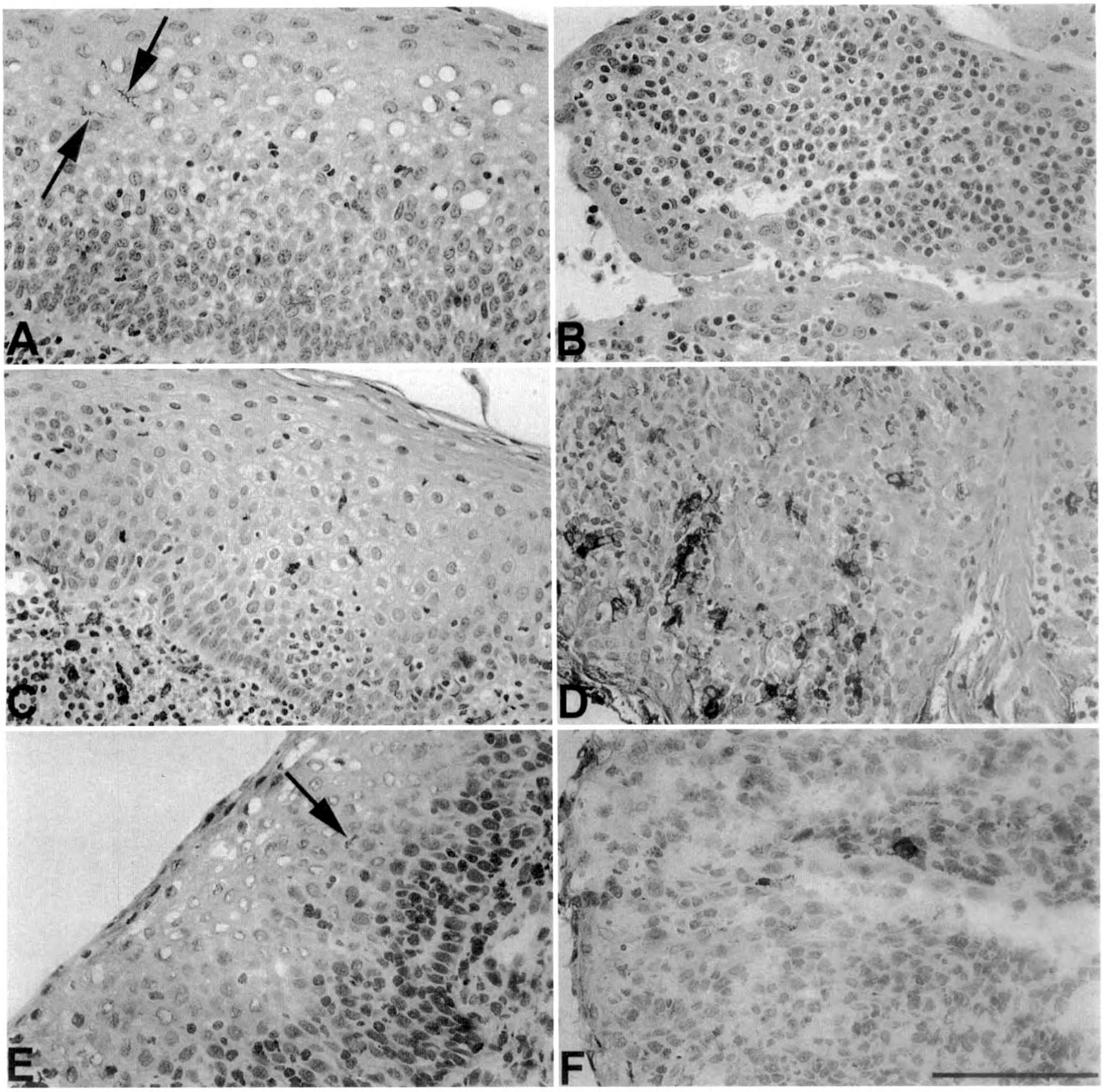


Fig 3. Surface (A,C,E) and crypt (B,D,F) epithelia of diseased tonsils (original ×400). Bar — 100 µm. A,B) CD1a staining of Langerhans cells. Arrows — positive cells. C,D) CD68 staining of macrophages. E,F) RFD7 staining of mature macrophages. Arrow — positive cell.

derstand disease processes in recurrent tonsillitis and tonsillar hyperplasia. This study has shown that both dendritic cells and macrophages reside within normal tonsil tissue. With inflammation and disease, those cell populations may undergo changes in quantity, location, and phenotype.

The family of dendritic cells was found to be the predominant type of antigen-presenting cell in the surface epithelium of normal tonsils. Included within this population are Langerhans cells (S-100, CD1a), indeterminate cells (S-100, CD1a), and interdigitating cells (S-100). Indeterminate cells differ from Langerhans cells in lacking Birbeck granules, and are thought to be precursors to them.[13,15] Expression of antigens recognized by RFD1 is associated with activation and antigen-presenting capacity.[40,41] Approximately half of the dendritic cells in the surface epithelium of normal tonsils were Langerhans cells that also bore antigens recognized by RFD1. The coexpression of RFD1 with CD1a suggested that those cells were capable of presenting antigen. Because RFD1-positive cells outnumbered those that expressed the Langerhans cell marker CD1a in the surface epithelium, half of those RFD1-positive cells with dendritic morphology were probably interdigitating cells.

The phenotypes of the dendritic cells in the dis-

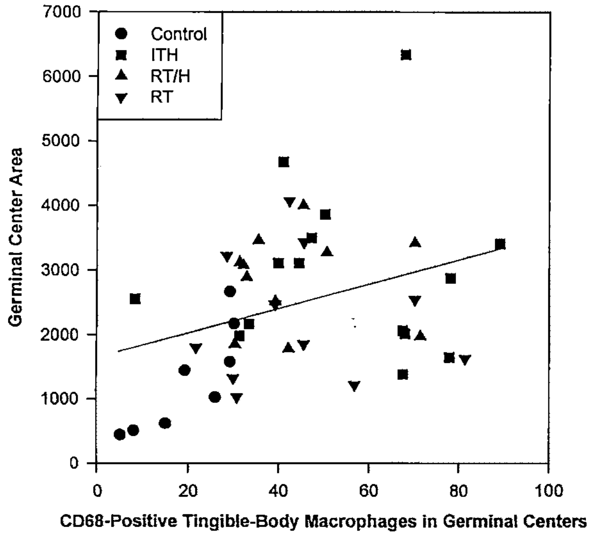


Fig 4. Germinal center size versus number of CD68-positive macrophages in germinal centers (r = .606). Diagonal line is regression generated statistically from these data. ITH — idiopathic tonsillar hyperplasia, RT/H — recurrent tonsillitis with persistent obstructive hyperplasia, RT — recurrent tonsillitis.

eased tonsils differed from those in the normal tonsils. Decreased expression of the RFD1 antigen in the surface epithelium of the diseased tonsils was presumptive evidence of reduced functional activation in disease. Langerhans cell numbers (CD1a) decreased with disease, as well. A similar phenomenon was found in the crypt epithelium. Although dendritic cells that expressed antigens recognized by RFD1 were observed in the crypt epithelium, an accurate assessment of the maturity and activation state of those cells could not be made because of concomitant B cell staining.

Although macrophages (CD68) were present in the surface epithelium of the normal tonsils, they were few in number. Widespread expression of the RFD7 phenotype attested to the maturity and activation of those macrophages. Macrophages were more numerous in the crypt than in the surface epithelium of normal tonsils. A smaller proportion of crypt macrophages appeared to be fully mature and/or activated (RFD7).

The phenotype of macrophages in diseased tonsils differed from that in normal tonsils. Despite increased numbers of CD68-positive macrophages in both the surface and crypt epithelia, there was not a concomitant rise in the expression of the antigens recognized by RFD7. That discrepancy provides strong circumstantial evidence that the majority of those macrophages were not mature and/or activated.

These phenotypic differences between dendritic cells and macrophages in normal and diseased tonsils give credence to earlier speculation about the possible contribution of local immunosuppression to chronic tonsillar disease.[10,22] In hyperplastic tonsils, although increased numbers of T cells and B cells were correlated with increased bacterial load and increased tonsillar volume, those lymphocytes were not fully immunocompetent and were relatively refractory to antigen stimulation in vitro. It is quite possible that the mechanism(s) responsible for decreased lymphocyte immunocompetence also accounts for reduced antigen-presenting cell activation.

It has been proposed that chronic infection could contribute to local immunosuppression by altering the tonsillar epithelium.[42] In that view, chronic or recurrent clinical infection might reflect a breakdown in the local "gatekeeper" functions of the tonsillar epithelium, including antigen presentation.[9] A primary function of the normal healthy crypt epithelium may be to provide a favorable environment for the requisite interaction among lymphocytes, antigen-presenting cells, and antigen to initiate vigorous and effective immunity.[6] Korsrud and Brandtzaeg[43] have proposed that the crypt epithelium functions as an additional lymphoid compartment, crucial to the protection of the mucosal surface of the tonsil. Repeated inflammation was shown to produce a metaplastic crypt epithelium, with decreased antigen receptor M cells, and decreased capacity for antigen uptake and processing.[44] It remains to be determined what effect this metaplastic epithelium might have on the antigen-presenting cells that normally reside within it. In previous studies from this laboratory, it was found that diseased tonsils exhibit an increased incidence of cryptitis and hyperkeratosis.[11] It has been shown that as the bacterial concentration of the dominant pathogen increases, so does the number of S-100–positive cells. However, those dendritic cells may not be mature, activated cells capable of processing antigen and presenting it to T cells.

In summary, increased margination of macrophages and increased numbers of lymphocytes attest to the recruitment of immune cells into diseased tonsils. The phenotypes of those cells suggest strongly that they are poorly functional, lacking characteristics associated with activation and functional maturity. For more direct assessment of the functional capacity of macrophages and dendritic cells, future studies will focus on measurements in vitro, comparing cells derived from normal and diseased tonsils. It remains to be determined whether tonsillar immune dysfunction results from an overwhelming bacterial load.

ACKNOWLEDGMENT — We thank the histology laboratory at the State University of New York at Buffalo, School of Medicine and Biomedical Sciences, for tissue processing and staining of the tonsillar tissues.

REFERENCES

1. Brandtzaeg P. Immune functions and immunopathology of palatine and nasopharyngeal tonsils. In: Bernstein J, Ogra P, eds. Immunology of the ear. New York, NY: Raven Press, 1987: 63-106.

2. Howie AJ. Scanning and transmission electron microscopy on the epithelium of human palatine tonsils. J Pathol 1980; 130:91-8.

3. von Gaudecker B, Muller-Hermelink HK. The development of the human tonsilla palatina. Cell Tissue Res 1982;224: 579-600.

4. Favre A, Paoli D, Poletti M, et al. The human palatine tonsil studied from surgical specimens at all ages and in various pathological conditions. Z Mikrosk Anat Forsch (Leipzig) 1986;100:7-33.

5. Perry ME, Jones MM, Mustafa Y. Structure of the crypt epithelium in human palatine tonsils. Acta Otolaryngol Suppl (Stockh) 1988(suppl 454):53-9.

6. Perry ME. The specialized structure of crypt epithelium in the human palatine tonsil and its functional significance. J Anat 1994;185:111-27.

7. Okato S, Magari S, Yamamoto Y, Sakanaka M, Takahashi H. An immuno-electron microscope study on interactions among dendritic cells, macrophages and lymphocytes in the human palatine tonsil. Arch Histol Cytol 1989;53:231-40.

8. Guidos C, Sinha AA, Lee C. Functional differences and complementation between dendritic cells and macrophages in T-cell activation. Immunology 1987;61:269-76.

9. Brodsky L, Moore L, Stanievich J. The role of *Haemophilus influenzae* in the pathogenesis of tonsillar hypertrophy in children. Laryngoscope 1988;98:1055-60.

10. Brodsky L, Moore L, Stanievich J, Ogra P. The immunology of tonsils in children: the effect of bacterial load on the presence of B and T subsets. Laryngoscope 1988;98:93-8.

11. Brodsky L, Frankel S, Gorfien J, Rossman J, Noble B. The role of dendritic cells in the development of chronic tonsillar disease in children. Acta Otolaryngol Suppl (Stockh) 1996 (suppl 523):98-100.

12. van der Valk P, van der Loo EM, Jansen J, Daha MR, Meijer CJLM. Analysis of lymphoid and dendritic cells in human lymph node, tonsil, and spleen. Virchows Arch (Cell Pathol) 1984;45:169-85.

13. Wood GS, Turner RR, Shiruba RA, Eng L, Warnke RA. Human dendritic cells and macrophages. Am J Pathol 1985;119: 73-82.

14. McMillan EM, Stoneking L, Humphrey GB, Rapacz J. Tonsillar mapping of determinants found on normal lymphoreticular (T, B, K, immature and macrophage) and myeloblastic leukemia cells. Am J Pathol 1987;126:452-63.

15. Weinberg DS, Pinkus GS, Murphy GF. Tonsillar epithelial dendritic cells. Lab Invest 1987;56:622-8.

16. Yamamoto Y, Okato S, Takahashi H, Takeda K, Magari S. Distribution and morphology of macrophages in palatine tonsils. Acta Otolaryngol Suppl (Stockh) 1988(suppl 454):83-95.

17. Yamamoto Y, Okato S, Nishiyama M, Takahashi H. Function and morphology of macrophages in palatine tonsils. Adv Otorhinolaryngol 1992;47:107-13.

18. Gloghini A, Carbone A. The nonlymphoid microenvironment of reactive follicles and lymphomas of follicular origin as defined by immunohistology on paraffin-embedded tissues. Hum Pathol 1993;24:67-76.

19. Ruco LP, Uccini S, Stoppacciaro A, et al. The lymphoepithelial organization of the tonsil: an immunohistochemical study in chronic recurrent tonsillitis. J Pathol 1995;176:391-8.

20. Tang X, Hori S, Osamura RY, Tsutsumi Y. Reticular crypt epithelium and intraepithelial lymphoid cells in the hyperplastic human palatine tonsil: an immunohistochemical analysis. Pathol Int 1995;45:34-44.

21. Crocker J. The enzyme histochemistry of lymphoid and non-lymphoid cells of the human palatine tonsil: a basis for the study of lymphomas. J Pathol 1981;134:81-95.

22. Hart DNJ, Starling GC, Calder VL, Fernando NS. B7/BB-1 is a leucocyte differentiation antigen of human dendritic cells induced by activation. Immunology 1993;79:616-20.

23. Spiteri MA, Poulter LW. Characterization of immune inducer and suppressor macrophages from the normal lung. Clin Exp Immunol 1991;83:157-62.

24. Poulter LW, Campbell DA, Munro C, Janossy G. Discrimination of human macrophages and dendritic cells by means of monoclonal antibodies. Scand J Immunol 1986;24:351-7.

25. Davis AL, McKenzie JL, Hart DNJ. HLA-DR–positive leucocyte subpopulations in human skin include dendritic cells, macrophages, and CD7-negative T cells. Immunology 1988;65: 573-81.

26. Alegre VA, MacDonald DM, Poulter LW. The simultaneous presence of Langerhans cell and interdigitating cell antigenic markers on inflammatory dendritic cells. Clin Exp Immunol 1986;64:330-3.

27. Frank G, Gorfien J, Mendel S, D'Auria P, Brodsky L, Noble B. Comparison of staining methods to detect interleukin-1β on antigen presenting cells and surface epithelial cells in the tonsil. J Histotechnol 1999;22:97-102.

28. Tacha DE, Cham T. Technical note: modified technique for microwave ovens. J Histotechnol 1994;17:365-6.

29. Flavell DJ, Jones DB, Wright DH. Identification of tissue histiocytes on paraffin sections by a new monoclonal antibody. J Histochem Cytochem 1987;35:1217-26.

30. Brandtzaeg P, Jones DB, Flavell DJ, Fagerhold MK. Monoclonal antibody to a human macrophage marker (MAC387) detects the formalin-resistant myelomonocytic L1 antigen. J Clin Pathol 1988;41:963-70.

31. Pulford KAF, Rigney EM, Micklem KJ, et al. KP1: a new monoclonal antibody that detects a monocyte/macrophage associated antigen in routinely processed tissue sections. J Clin Pathol 1989;42:414-21.

32. Micklem K, Rigney E, Cordel J, et al. A human macrophage-associated antigen (CD68) detected by six different monoclonal antibodies. Br J Haematol 1989;73:6-11.

33. Saito N, Pulford KAF, Breton-Gorios J, Masse JM, Mason DY, Cramer EM. Ultrastructural localization of the CD68 macrophage-associated antigen in human blood neutrophils and monocytes. Am J Pathol 1991;139:1053-9.

34. Krenacs L, Tiszalvicz L, Krenacs T, Boumsell L. Immunohistochemical detection of CD1a antigen in formalin-fixed and paraffin-embedded tissue sections with monoclonal antibody 010. J Pathol 1993;171:99-104.

35. Emile JF, Wechsler J, Brousse N, et al. Langerhans cell

histiocytosis. Am J Surg Pathol 1995;19:636-41.

36. Kindblom LG, Lodding P, Rosengren L, Baudier J, Haglid K. S-100 protein in melanocytic tumors. Acta Path Microbiol Immunol (Scand Sect A) 1984;92:219-30.

37. Adams TE, Bodmer JG, Bodmer WF. Production and characterization of monoclonal antibodies recognizing the α-chain subunits of human 1a alloantigens. Immunology 1983;50: 613-24.

38. Gorfien JL, Hard R, Noble B, Brodsky L. Quantitative study of germinal center area in normal and diseased tonsils using image analysis. Ann Otol Rhinol Laryngol 1999;108:398-402.

39. Tabe H, Kawabata I, Koba R, Homma T. Cell dynamics in the germinal center of the human tonsil. Acta Otolaryngol Suppl (Stockh) 1996(suppl 523):64-7.

40. Buckley CC, Ivison C, Poulter LW, Rustin MHA. FcεR11/CD23 receptor distribution in patch test reactions to aeroallergens in atopic dermatitis. J Invest Dermatol 1992;99:184-8.

41. Bos JD, van Garderen ID, Krieg SR, Poulter LW. Different in situ distribution patterns of dendritic cells having Langerhans (T6+) and interdigitating (RFD1+) cell immunophenotype in psoriasis, atopic dermatitis, and other inflammatory dermatoses. J Invest Dermatol 1986;87:358-61.

42. Koch RJ, Brodsky L. Effect of specific bacteria on lymphocyte proliferation in diseased and nondiseased tonsils. Laryngoscope 1993;103:1020-6.

43. Korsrud FR, Brandtzaeg P. Immunohistochemical evaluation of J-chain expression by intra- and extra-follicular immunoglobulin-producing human tonsillar cells. Scand J Immunol 1981;13:271-80.

44. Surján L. Reduced lymphocyte activation in repeatedly inflamed human tonsils. Acta Otolaryngol (Stockh) 1980;89: 187-94.

* * *

MANAGEMENT OF THE TINNITUS PATIENT

The Ninth Annual Conference on the Management of the Tinnitus Patient, for professionals and tinnitus patients, will be held in Iowa City, Iowa, October 4-6, 2001. For information, see web site www.medicine.uiowa.edu/otolaryngology/news/news or contact Rich Tyler by telephone (319-356-2471) or e-mail (rich-tyler@uiowa.edu).

Ann Otol Rhinol Laryngol 110:2001

QUANTITATIVE ASSESSMENT OF THE PHARYNGEAL AIRWAY BY DYNAMIC MAGNETIC RESONANCE IMAGING IN OBSTRUCTIVE SLEEP APNEA SYNDROME

KATSUHISA IKEDA, MD
MASAKI OGURA, MD
TAKESHI OSHIMA, MD
HIDEAKI SUZUKI, MD
SHUICHI HIGANO, MD
SHOKI TAKAHASHI, MD
HAJIME KUROSAWA, MD
WATARU HIDA, MD
HIROO MATSUOKA, MD
TOMONORI TAKASAKA, MD
SENDAI, JAPAN

Dynamic changes in the pharyngeal airway of patients with obstructive sleep apnea syndrome (OSAS) were evaluated by quantitating the findings of real-time imaging performed during wakefulness and spontaneous sleep by means of dynamic magnetic resonance imaging (MRI). Six patients with OSAS and 3 non-OSAS subjects, selected prospectively and randomly, underwent polysomnography and dynamic MRI. The cross-sectional areas of the soft palate and oropharynx and the anterior-posterior airway dimensions seen during wakefulness and spontaneous sleep were calculated by US National Institutes of Health imaging software. On the basis of a case control study, comparisons were made with age-matched and body mass index–matched obese non-OSAS snorers. Spontaneous sleep caused significant obstruction and narrowing of various sites of the pharyngeal airway in the OSAS patients, but not in the non-OSAS subjects. During wakefulness, the non-OSAS subjects showed no marked narrowing of the pharyngeal airways, whereas a transient but significant narrowing was observed in the OSAS patients. The mean values of both the cross-sectional area and the anterior-posterior diameter at the soft palate were significantly reduced by spontaneous sleep in the OSAS patients. Dynamic MRI in awake OSAS patients shows promise as a routine diagnostic tool for localizing the upper airway collapse for appropriate selection of surgical therapy.

KEY WORDS — dynamic magnetic resonance imaging, pharyngeal airway, polysomnography, sleep apnea syndrome.

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is defined as intermittent, complete or partial upper airway obstruction that occurs during sleep and causes mental and physical effects.[1] The main symptoms of OSAS include loud and irregular snoring, breathing pauses, restless sleep, and daytime sleepiness.[2] Abnormalities of the structure and function of the upper airway have been established as causative factors in OSAS. Patients with OSAS frequently have airway collapse at different sites from the oropharynx to the hypopharynx.

Understanding of the upper airway structure and function in OSAS patients is needed for insight into the pathogenesis of this disorder and to improve the selection of therapy and the success rate of the surgical treatment for OSAS.[3,4] The localization of obstructions in the upper airways in OSAS patients has been studied by a variety of methods, including physical examination, simple fiberoptic endoscopy,[5] fiberoptic endoscopy with the so-called Müller maneuver,[6] pharyngeal pressure measurements,[7-10] cephalometrics,[11] computed tomography,[12,13] and fluoroscopy.[14,15] Most of these methods have been performed in awake patients.

Recently, it has become possible for magnetic resonance imaging (MRI) to determine accurately the upper airway cross-sectional area and volume.[16-19] Since the cross-sectional area of the upper airway varies with respiration,[12,20-24] total occlusion and abrupt narrowing are overlooked or not fully appreciated by some diagnostic imaging methods, which lack sufficient temporal resolution.[25-28] Large changes in the pharyngeal cross-sectional area with respiration could perhaps give erroneous images on conventional MRI. The development and implementation of dynamic MRI for the assessment of OSAS has provided useful information on structural alterations in the pharyngeal airway, the location of abnormal sites, and the severity of apnea.[23-30] However, no previous reports have described a real-time quantitative analysis of the cross-sectional area of the upper airway performed during spontaneous sleep.[28-35]

In this study, we applied dynamic MRI to carefully evaluate the pharyngeal airway by quantitatively calculating the findings of real-time imaging per-

---

From the Departments of Otorhinolaryngology (Ikeda, Ogura, Oshima, Suzuki, Takasaka), Diagnostic Radiology (Higano, Takahashi), Internal Medicine (First Department; Kurosawa, Hida), and Neuropsychiatry (Matsuoka), Tohoku University School of Medicine, Sendai, Japan.

CORRESPONDENCE — Katsuhisa Ikeda, MD, Dept of Otorhinolaryngology, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan.

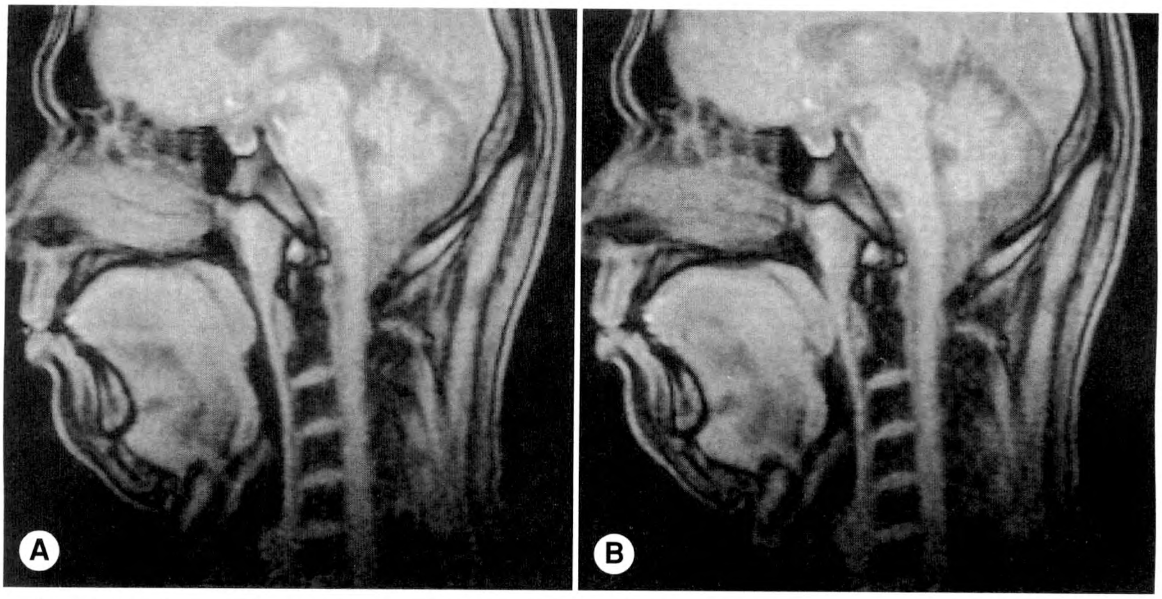


Fig 1. Sagittal magnetic resonance images of representative patient with obstructive sleep apnea syndrome (OSAS). A) Awake. B) Asleep.

formed during wakefulness and spontaneous sleep in patients with OSAS. Moreover, these data obtained from OSAS patients were compared with those of obese non-OSAS snorers matched for age and body mass index (BMI).

MATERIALS AND METHODS

Patients. Nineteen patients who complained of sleep apnea and snoring were prospectively and randomly selected from among those attending the Department of Otolaryngology, Tohoku University School of Medicine, and were enrolled in the MRI study. Six patients (5 men and 1 woman; mean age, 43 years; range, 35 to 64 years) with OSAS and 3 non-OSAS snorers matched for age (3 men; mean age, 36 years; range, 27 to 57 years) were eligible for the study. The remaining 13 subjects were unable to complete spontaneous sleep during MRI because of the presence of the background noise of MRI. The diagnosis of OSAS was established by a combination of medical history, clinical examination, and standard nocturnal polysomnography. Informed consent was obtained from all patients, and the study was approved by the Ethics Committee of Tohoku University School of Medicine.

Polysomnography. The overnight sleep study was performed in a quiet, darkened room by standard polysomnographic techniques as described previously,[36] including electroencephalography, electro-oculography, submental electromyography with surface electrodes, airflow at the nose and mouth obtained with 2 thermistors, tracheal sound obtained with a microphone, inductive plethysmography (Respitrace, Ambulatory Monitoring, Ardsley, NY), and percutaneous arterial oxygen saturation (SpO_2) obtained with a finger pulse oximeter (Biox 3700, Ohmeda, Boulder, Colo). All variables were continuously recorded on a multichannel thermal polygraph (Rectihoriz-8K, NEC San-ei, Tokyo, Japan) and a data recorder (A-109, Sony, Tokyo).

Apnea was defined as a cessation of airflow at the nose and mouth lasting longer than 10 seconds. Hypopnea was defined as a more than 50% reduction in thoracoabdominal amplitude (Respitrace sum signals) associated with a reduction in SpO_2 of more than 4% from the preceding values. The apnea-hypopnea index (AHI) was calculated from episodes of apnea and hypopnea per hour of sleep.

Magnetic Resonance Imaging. The MRI was performed with a 1.5-T superconducting system (Magneton Vision, Siemens Medical Systems, Erlangen, Germany) combined with a neck coil. Each patient was placed in the supine position, and the neck angle was fixed. The patients underwent MRI when they were spontaneously asleep and awake. Diurnal sleep during MRI was monitored by observing the thoracoabdominal respiratory motion with an MRI-linked cuff for respiratory synchronization and measuring SpO_2. However, stages of sleep were not analyzed during MRI. Images were obtained in 2 views — the median sagittal view and the axial view — at the levels of both the narrowest part of the soft palate (just above the lower tip of the uvula) and the narrowest part of the oropharynx (between the lower tip of the uvula and the upper tip of the epiglottis). The image sequence was an inversion-recovery turbo fast low angle shot sequence (repetition time, 4.5 ms; echo time, 2.2 ms; inversion time, 500 ms; flip angle, 8°;

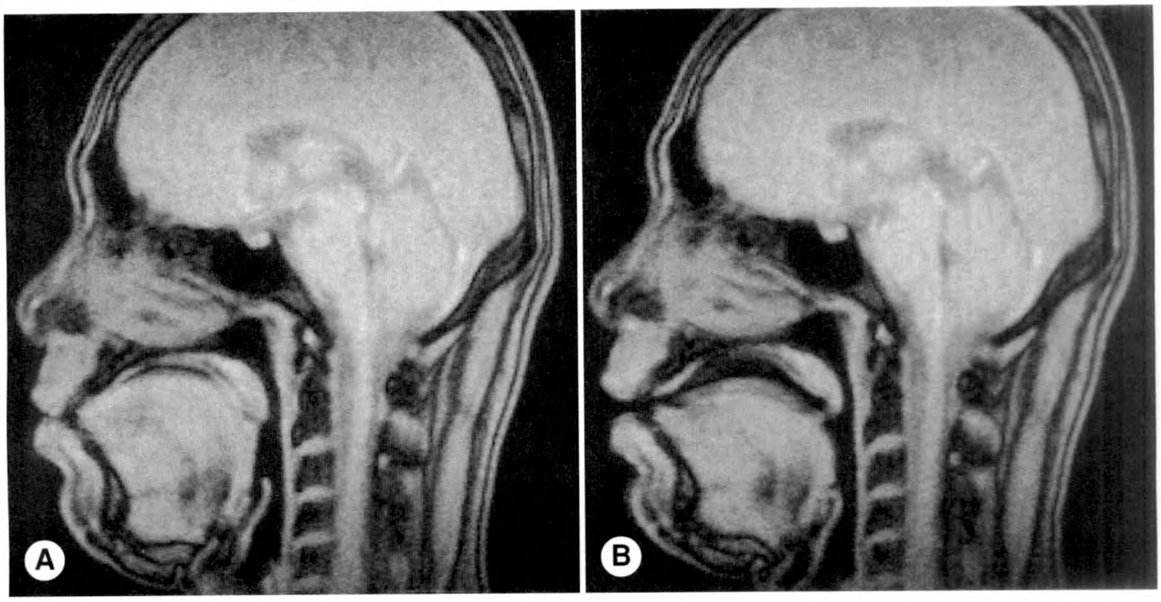


Fig 2. Sagittal magnetic resonance images of representative non-OSAS subject. A) Awake. B) Asleep.

field of view, 250 mm; matrix, 128 × 12; section thickness, 9 mm). The imaging time per slice for this sequence was 0.8 second. Each sagittal and axial view was obtained as a single section. A total of 50 images was serially obtained at each section. To assess the relative accuracy of dynamic MRI for measuring cross-sectional areas, we conducted a phantom study. The phantom contained cylinders of various sizes. Cross-sectional areas were determined for these cylinders by means of a cursor and the software incorporated with the MRI scanner. The accuracy of the measurement was ±5%.

Raw data of all magnetic resonance images were directly transferred to a personal computer (Apple Power Macintosh 4400/200), and were processed on the computer with image analyzing software (NIH Image version 1.58).[37] To fit the limitation of the software (8 bits pixel value), 12 bits of MRI pixel data were converted to 8 bits at a scale factor of 0.5 at the minimum (pixel value, 144) and maximum (pixel value, 650) levels. The airway measurements made included cross-sectional area and anterior-posterior (A-P) width.

Statistical Analysis. The results were expressed as mean ± SD and were compared by a paired or unpaired *t*-test; a p value of less than .05 was recognized as significant.

RESULTS

The BMI (kg/m^2) was 27.6 ± 2.1 (mean ± SD) for OSAS patients and 25.2 ± 0.5 for non-OSAS snorers. The AHI in non-OSAS snorers was less than 10 (average, 2.9 ± 4.5), whereas OSAS patients showed

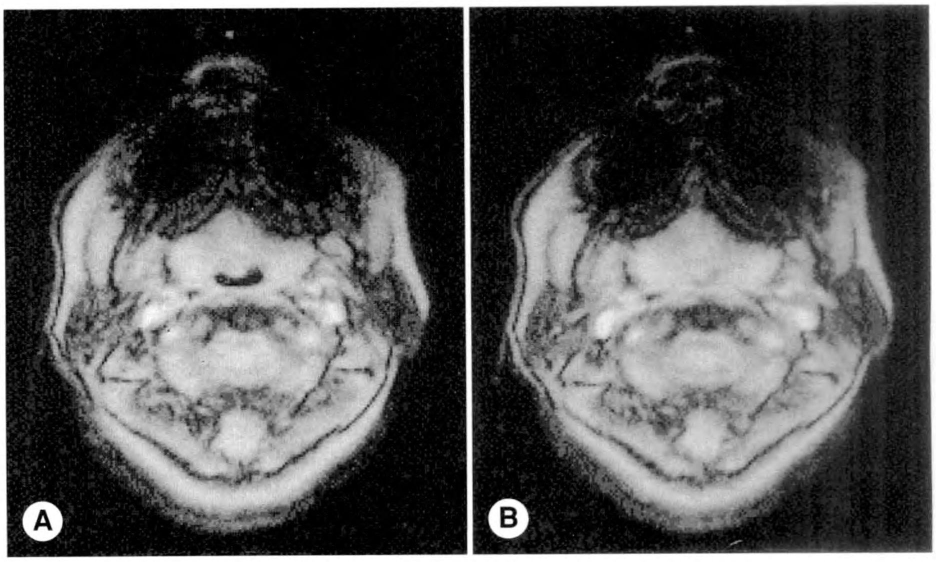


Fig 3. Axial magnetic resonance images at soft palate of representative OSAS patient. A) Awake. B) Asleep.

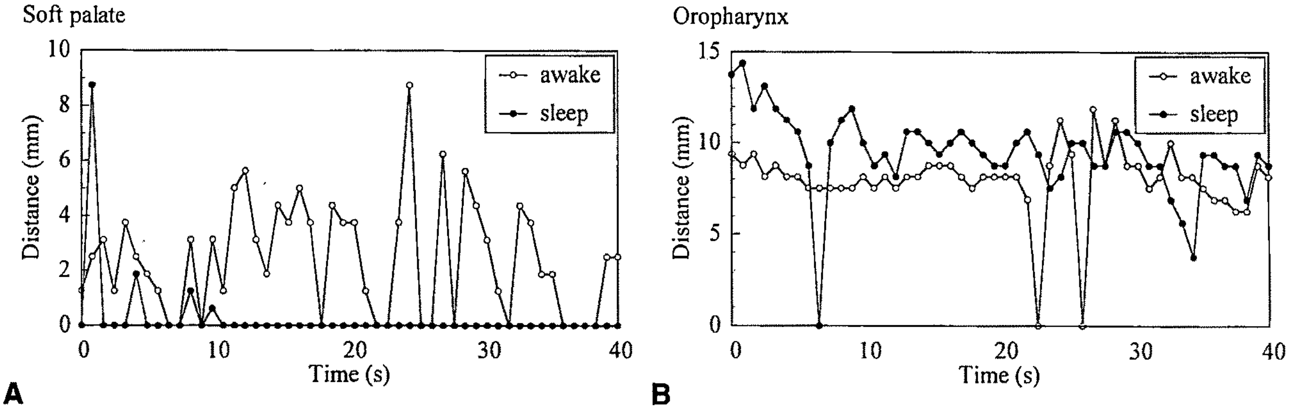


Fig 4. Time course of anterior-posterior (A-P) diameter at A) soft palate and B) oropharynx of representative OSAS patient awake and asleep.

an average AHI of 35.9 ± 13.9.

On the sagittal magnetic resonance images, all 6 OSAS patients showed obstructions of the pharyngeal airway at the soft palate during spontaneous sleep (Fig 1), whereas obstructions of the oropharynx were observed in 2 OSAS patients. On the other hand, no complete obstruction was observed in obese non-OSAS snorers (Fig 2). Similarly, the axial views demonstrated obstruction of the pharyngeal airway at the soft palate in the OSAS patients during sleep (Fig 3).

The time courses of the A-P diameters and the cross-sectional areas at the levels of the soft palate and oropharynx during awake and asleep conditions in OSAS patients and non-OSAS subjects were obtained from the magnetic resonance images. In 5 of the 6 OSAS patients, a transient but marked narrowing of the pharyngeal airway at the soft palate was seen during awake tidal breathing. A complete obstruction was observed in all OSAS patients during spontaneous sleep (Figs 4 and 5). In contrast, no marked narrowing was seen during wakefulness in the non-OSAS subjects. A slight narrowing without obstruction was seen in all non-OSAS subjects during sleep (Figs 6 and 7). Figure 8 shows the average values of the A-P diameters and the cross-sectional areas of the pharyngeal airway. There was no difference ($p > .05$) between OSAS patients and non-OSAS snorers in the mean cross-sectional areas at the soft palate and the oropharynx during wakefulness. In non-OSAS patients, neither the mean cross-sectional areas nor the A-P diameters during sleep were significantly different ($p > .05$) from those obtained during wakefulness. On the other hand, in OSAS patients, spontaneous sleep significantly decreased the mean cross-sectional area ($p = .02$) and the mean A-P diameter ($p = .04$) at the soft palate, but not those at the oropharynx ($p > .05$).

DISCUSSION

We used dynamic MRI to assess abnormal changes in the pharyngeal airway dimensions. Previous studies have documented that rapid imaging techniques are essential for a thorough and accurate assessment of the pharyngeal airway of OSAS patients.[25-28] Occlusion and narrowing are most apparent during tidal breathing because of the dynamic alterations in the pharyngeal airway associated with changes in lung volume.[12,20-24] Therefore, rapid imaging can detect

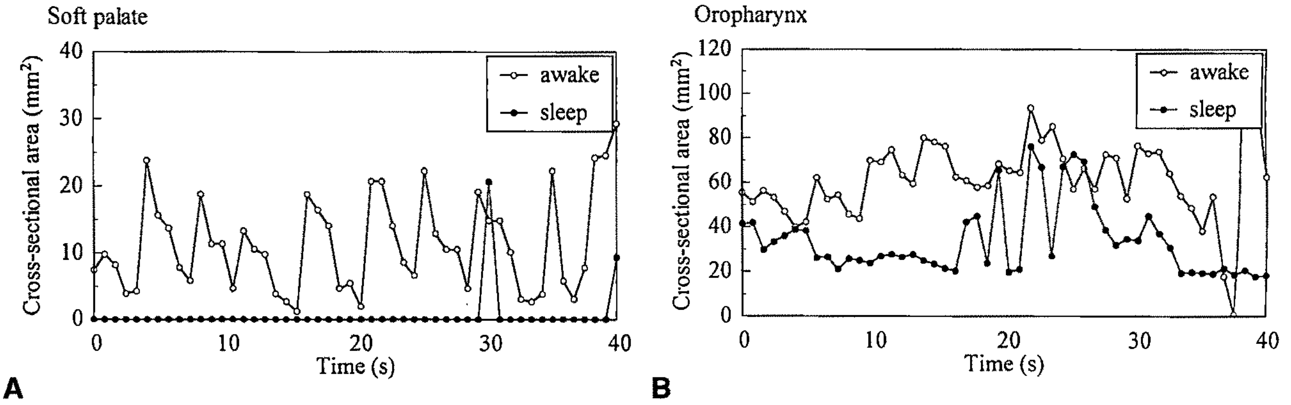


Fig 5. Time course of cross-sectional area at A) soft palate and B) oropharynx of representative OSAS patient awake and asleep.

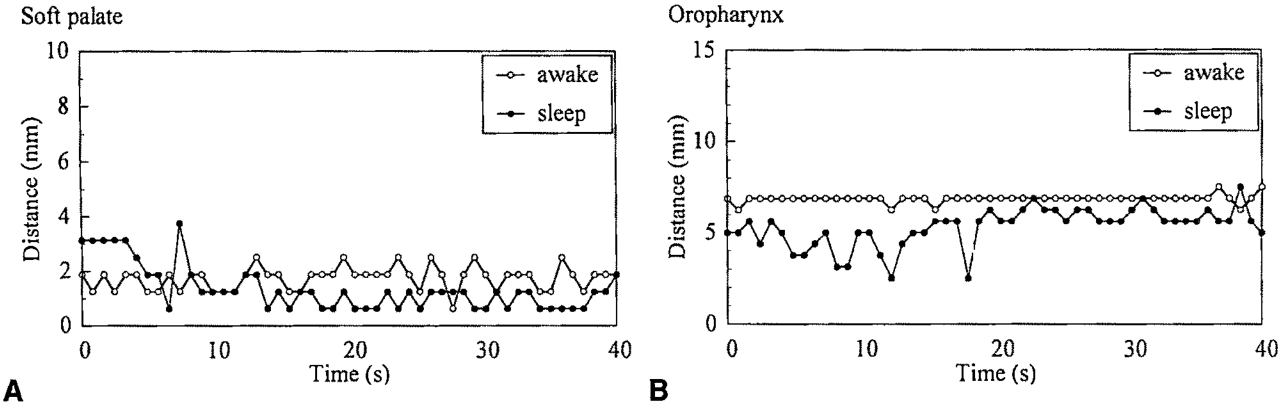


Fig 6. Time course of A-P diameter at A) soft palate and B) oropharynx of representative non-OSAS subject awake and asleep.

the morphological abnormalities of the pharyngeal airway of OSAS patients, which are often transient. The advantages of MRI of the pharyngeal airway, compared with cine computed tomography, include the absence of exposure to ionizing radiation, adequate time and spatial resolution, a lower chance of artifacts associated with metallic dental objects, the absence of a partial volume effect, and the easy availability of sagittal images. Another approach to monitoring the pharyngeal airway is to use a fiberoptic catheter. However, the premedication or local anesthesia used would alter the sleep architecture and the physiological behavior of the upper airways.

The present study on dynamic MRI is the first, though preliminary, demonstration that spontaneous sleep significantly causes an obstruction and narrowing of the upper airway in OSAS patients, but not in age- and BMI-matched non-OSAS snorers. So far, the evaluation of the upper airway in OSAS patients has been undertaken in artificial, drug-induced sleep.[29-35] Our purpose was to evaluate the upper airway architecture in spontaneous diurnal sleep without interference from the problems that could be induced by sedatives, such as severe apnea,[38] a decrease in genioglossal muscle activity,[39] and depression of the central nervous system.[38] Imaging could only be obtained from a limited number of OSAS patients and non-OSAS snorers, since the noise generated by the MRI system often disturbed the spontaneous sleep. The present results, which are still preliminary, confirm that spontaneous sleep actually causes a marked collapse of various sites of the pharyngeal airway. Further, an abnormally but transiently narrowed pharyngeal airway was observed in awake OSAS patients that was not apparent in non-OSAS snorers. Patients with OSAS are known to have smaller pharyngeal airways and higher pharyngeal airway resistance during wakefulness.[23-30] When these patients sleep, a state-related reduction in upper airway muscle activity removes the essential compensatory mechanisms, and airway collapse ensues as a result of inspiratory dynamic compression.[40] The present data, comparing the pharyngeal airways in both awake and asleep phases, appear to confirm that patients with OSAS have transiently narrowed pharyngeal airways during wakefulness. In other words, an evaluation of dynamic changes in the pharyngeal airway in wakefulness may suggest the sleeping condition.

Nasal continuous positive airway pressure has a potential success rate of up to 99%, constituting an extraordinary advance.[41,42] However, several reports have indicated unsatisfactory compliance due to the

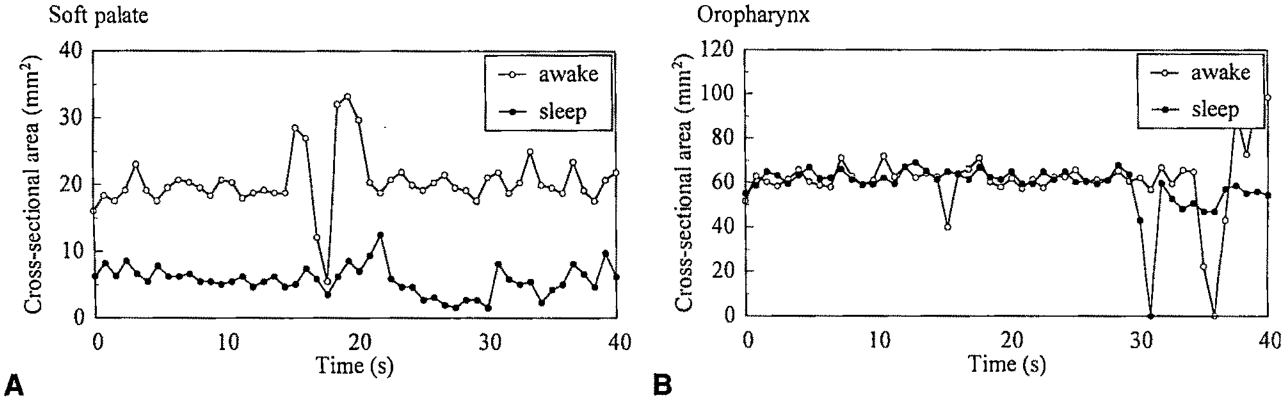


Fig 7. Time course of cross-sectional area at A) soft palate and B) oropharynx of representative non-OSAS subject awake and asleep.

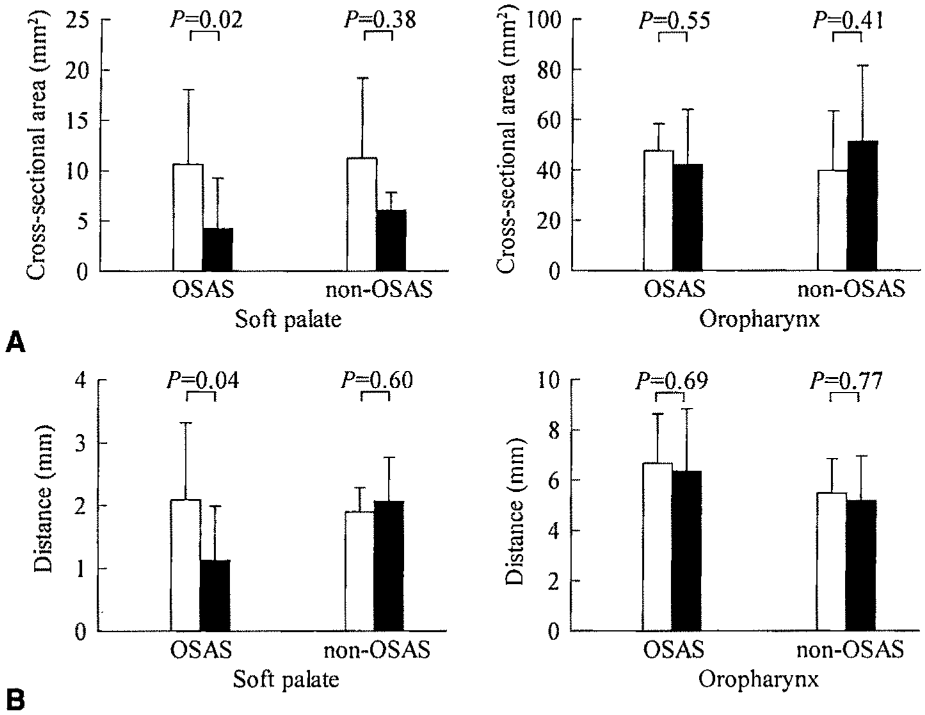


Fig 8. Comparison of mean airway measurements in non-OSAS subjects and OSAS patients awake (open columns) and asleep (closed columns). A) Cross-sectional area. B) A-P diameter.

cumbersome and uncomfortable nature of the device.[43-45] Many patients want to try surgical approaches for the treatment of OSAS. Unfortunately, the absence of diagnostic tools for localizing the obstruction makes it difficult to select an appropriate surgical therapy. The outcome of surgical therapy can be improved by determining the site of the upper airway collapse.[4,46] Use of MRI in wakefulness, and during sleep when possible, may provide useful data for the appropriate selection of surgical options. More clinical studies are necessary to determine whether data obtained from such MRI studies help to predict the success of surgical therapy.

In conclusion, dynamic MRI, by quantitating the real-time imaging, was used to assess the pharyngeal airway in patients with OSAS during wakefulness and spontaneous sleep, and these data were compared with those obtained in age- and BMI-matched non-OSAS snorers. Only in OSAS patients did spontaneous sleep result in occlusion or narrowing of various sites of the pharyngeal airway; they also had a degree of occlusion during wakefulness. Use of dynamic MRI in awake OSAS patients may be promising as a routine diagnostic tool for localizing the upper airway collapse to assist in the selection of appropriate surgical therapy.

REFERENCES

1. Guilleminault C, Tilkian A, Dement WC. The sleep apnea syndrome. Annu Rev Med 1976;27:465-84.
2. Whyte KF, Allen MB, Jeffrey AA, Gould GA, Douglas NJ. Clinical features of the sleep apnoea/hypopnoea syndrome. Q J Med 1989;72:659-66.
3. Katsantonis GP, Walsh JK. Somnofluoroscopy: its role in the selection of candidates for uvulopalato-pharyngoplasty. Otolaryngol Head Neck Surg 1986;94:56-60.
4. Ikeda K, Oshima T, Shimomura A, Takasaka T. Surgical criteria for obstructive sleep apnea syndrome based on localization of upper airway collapse during sleep: a preliminary study. Tohoku J Exp Med 1998;185:1-8.
5. Guilleminault C, Hill MW, Simmons FB, Dement WC. Obstructive sleep apnea: electromyographic and fiberoptic studies. Exp Neurol 1978;62:49-67.
6. Sher AE, Thorphy MJ, Shprintzen RJ, Spielman AJ, Burack B, McGregor PA. Predictive value of Muller maneuver in selection of patients for uvulopalatopharyngoplasty. Laryngoscope 1985;95:1483-7.
7. Issa FG, Sullivan CE. Upper airway closing pressures in obstructive apnea. J Appl Physiol 1984;57:520-7.
8. Shepard JW, Thawley SE. Localization of upper airway collapse during sleep in patients with obstructive sleep apnea. Am Rev Respir Dis 1990;141:1350-5.
9. Shepard JW, Gefter WB, Guilleminault C, et al. Evaluation of the upper airway in patients with obstructive sleep apnea. Sleep 1991;14:361-71.
10. Gleadhill IC, Schwartz AL, Schubert N, Wise RA, Permutt S, Smith PL. Upper airway collapsibility in snorers and in patients with obstructive hypopnea and apnea. Am Rev Respir Dis 1991;143:1300-3.
11. Riley RW, Powell NB, Guilleminault C, Nino-Murcia G. Maxillary, mandibular, and hyoid advancement: an alternative to tracheostomy in obstructive sleep apnea syndrome. Otolaryngol Head Neck Surg 1986;94:584-8.
12. Stein MG, Gamsu G, de Geer G, Golden JA, Crumley RL, Webb WR. Cine CT in obstructive sleep apnea. AJR Am J Roentgenol 1987;148:1069-74.
13. Horner RL, Shea SA, McIvor J, Guz A. Pharyngeal size

and shape during wakefulness and sleep in patients with obstructive sleep apnea. Q J Med 1989;72:719-35.

14. Smith TH, Baska RE, Francisco CB, McGray GM, Kunz S. Sleep apnea syndrome: diagnosis of upper airway obstruction by fluoroscopy. J Pediatr 1978;93:891-2.

15. Pepin JL, Ferretti G, Veale D, et al. Somnofluoroscopy, computerised tomography and cephalometry in the assessment of the airway on obstructive sleep apnoea. Thorax 1992;47:150-6.

16. Abbey NC, Block AJ, Green D, Mancuso A, Hellard DW. Measurement of pharyngeal volume by digitized magnetic resonance imaging. Am Rev Respir Dis 1989;140:717-23.

17. Ryan CF, Lowe AA, Li D, Fleetham JA. Magnetic resonance imaging of the upper airway in obstructive sleep apnea before and after chronic nasal continuous positive airway pressure therapy. Am Rev Respir Dis 1991;144:939-44.

18. Shelton KE, Woodson H, Gay S, Suratt PM. Pharyngeal fat in obstructive sleep apnea. Am Rev Respir Dis 1993;148:462-6.

19. Schwab RJ, Gupta KB, Gefter WB, Metzger LJ, Hoffman EA, Pack AI. Upper airway and soft tissue anatomy in normal subjects and patients with sleep-disordered breathing. Am J Respir Crit Care Med 1995;152:1673-89.

20. Ell SR, Jolles H, Galvin JR. Cine CT demonstration of nonfixed upper airway obstruction. AJR Am J Roentgenol 1986; 146:669-77.

21. Galvin JR, Rooholamini SA, Stanford W. Obstructive sleep apnea: diagnosis with ultrafast CT. Radiology 1989;171: 775-8.

22. Brown JG, Zamel N, Hoffstein V. Pharyngeal cross-sectional area in normal men and women. J Appl Physiol 1986;61: 890-5.

23. Stanford W, Galvin J, Rooholamini M. Effects of awake tidal breathing, swallowing, nasal breathing, oral breathing and the Muller and Valsalva maneuvers on the dimensions of the upper airway: evaluation by ultrafast computerized tomography. Chest 1988;94:149-54.

24. Brown IG, Zamel N, Hoffstein V. Pharyngeal cross-sectional area in normal men and women. J Appl Physiol 1986;61: 890-5.

25. Haponik EF, Smith PL, Bohlman ME, Allen RP, Goldman SM, Bleecker ER. Computerized tomography in obstructive sleep apnea: correlation of airway size with physiology during sleep and wakefulness. Am Rev Respir Dis 1983;127:221-6.

26. Suratt PM, Dee P, Atkinson RL, Armstrong P, Wilhoit SC. Fluoroscopic and computed tomographic features of the pharyngeal airway in obstructive sleep apnea. Am Rev Respir Dis 1983;127:487-92.

27. Rivlin F, Hoffstein V, Kalbfleisch J, McNicholas W, Zamel H, Bryan AC. Upper airway morphology in patients with idiopathic obstructive sleep apnea. Am Rev Respir Dis 1984; 129:355-60.

28. Shellock FG, Schatz CJ, Julien P, et al. Occlusion and narrowing of the pharyngeal airway in obstructive sleep apnea: evaluation by ultrafast spoiled GRASS MR imaging. AJR Am J Roentgenol 1992;158:1019-24.

29. Suto Y, Matsuo T, Kato T, et al. Evaluation of the pharyngeal airway in patients with sleep apnea: value of ultrafast MR imaging. AJR Am J Roentgenol 1993;160:311-4.

30. Suto Y, Matsuda E, Inoue Y, Suzuki T, Ohta Y. Sleep apnea syndrome: comparison of MR imaging of the oropharynx with physiological indexes. Radiology 1996;201:393-8.

31. Suto Y, Inoue Y. Sleep apnea syndrome. Examination of pharyngeal obstruction with high-speed NR and polysomnography. Acta Radiol 1996;37:315-20.

32. Okada T, Fukatsu H, Ishigaki T, Yasuma F, Kayukawa Y. Ultra-low-field magnetic resonance imaging in upper airways obstruction in sleep apnea syndrome. Psychiatry Clin Neurosci 1996;50:285-9.

33. Suto Y, Matsuda E, Inoue Y. MRI of the pharynx in young patients with sleep disorder breathing. Br J Radiol 1996;69: 1000-4.

34. Yokoyama M, Yamanaka N, Ishii H, Tamaki K, Yoshikawa A, Morita R. Evaluation of the pharyngeal airway in obstructive sleep apnea: study by ultrafast MR imaging. Acta Otolaryngol Suppl (Stockh) 1996(suppl 523):242-4.

35. Hasegawa K, Nishimura T, Yahisawa M, Morishima N, Shibata N, Iwanaga K. Diagnosis by dynamic MRI in sleep disordered breathing. Acta Otolaryngol Suppl (Stockh) 1996(suppl 523):245-7.

36. Hida W, Okabe S, Miki H, et al. Effects of submental stimulation for several consecutive nights in patients with obstructive sleep apnoea. Thorax 1994;49:446-52.

37. Lenard P. Image analysis for all. Nature 1990;347:103-4.

38. Mendelson WB, Garnett D, Gillin JC. Flurazepam-induced sleep apnea syndrome in a patient with insomnia and mild sleep-related respiratory changes. J Nerv Ment Dis 1981; 169:261-4.

39. Leiter JC, Knuth SL, Krol RC, Bartlett D Jr. The effect of diazepam on genioglossal muscle activity in normal human subjects. Am Rev Respir Dis 1985;132:216-9.

40. Leiter JC. Upper airway shape. Is it important in the pathogenesis of obstructive sleep apnea? Am J Respir Crit Care Med 1996;153:894-8.

41. Phillips BA, Schmitt FA, Berry DR, Lamb DG, Anim M, Cook YR. Treatment of obstructive sleep apnea: a preliminary report comparing nasal CPAP to nocturnal oxygen in patients with mild OSA. Chest 1990;98:325-30.

42. Sullivan CE, Issa FG, Berthon-Jons M, Eves L. Reversal of obstructive sleep apnoea by continuous positive airway pressure through the nares. Lancet 1981;1:862-5.

43. Engleman HM, Douglas NJ. CPAP compliance. Sleep 1991;20:S114.

44. Hoffstein V, Viner S, Mateika S, Convay J. Treatment of obstructive sleep apnea with nasal continuous positive airway pressure. Patient compliance, perception of benefits, and side effects. Am Rev Respir Dis 1992;145:841-5.

45. Kribbs NB, Pack AI, Kline LR, et al. Objective measurement of patterns of nasal use by patients with obstructive sleep apnea. Am Rev Respir Dis 1993;147:887-95.

46. Launois SH, Feroah TR, Campbell WN, et al. Site of pharyngeal narrowing predicts outcome of surgery for obstructive sleep apnea. Am Rev Respir Dis 1993;147:182-9.

Ann Otol Rhinol Laryngol 110:2001

EFFECT OF CIGARETTE SMOKING ON GASTROPHARYNGEAL AND GASTROESOPHAGEAL REFLUX

CONRAD F. SMIT, MD
MARCEL P. COPPER, MD, PHD
JUSTIN A. M. J. VAN LEEUWEN, MD
IVO G. SCHOOTS, MD
LAKI D. STANOJCIC, MD
AMSTERDAM, THE NETHERLANDS

Gastropharyngeal reflux appears to be associated with various otolaryngological complaints. Cigarette smoking is known to affect adversely the defense mechanisms against reflux of acid gastric contents into the esophagus. To study the relationship between gastropharyngeal, as well as gastroesophageal, reflux and cigarette smoking, 15 subjects underwent 24-hour double-probe pH monitoring while smoking their daily amount of cigarettes. The percentage of time the pH was below 4 during the smoking period was significantly higher than the percentage of time the pH was below 4 during the nonsmoking period, proximal, at the level of the upper esophageal sphincter, as well as distal, above the lower esophageal sphincter. These findings demonstrate that smoking increases gastropharyngeal and gastroesophageal reflux. Smokers with complaints and disorders caused by reflux should therefore be advised to stop smoking in order to reduce reflux.

KEY WORDS — acid, cigarette smoking, gastroesophageal reflux, gastropharyngeal reflux, pH monitoring.

INTRODUCTION

Cigarette smoking is supposed to increase gastroesophageal reflux (GER).[1-12] The effect of cigarette smoking on gastropharyngeal reflux (GPR), however, is less known. Under normal conditions, reflux of acid gastric fluid and its noxious effect are prohibited by the so-called antireflux barrier. The most important components of this barrier are the lower esophageal sphincter (LES) tone, in close connection to the influence of the crura of the diaphragm, esophageal acid clearance, esophageal epithelial resistance, and the upper esophageal sphincter (UES) tone.[13] Smoking decreases the LES pressure during the act of smoking.[1-3,6,7,12] Almost immediately after the start of smoking, the sphincter pressure falls, and it returns to normal within 8 minutes after the smoker finishes the cigarette.[1,2] Cigarette smoking also has a negative effect on esophageal acid clearance,[2,5,10] salivary output,[5] and gastric emptying.[8,9] Furthermore, smoking diminishes both the pharyngo-UES contractile reflex and the pharyngeal reflexive swallow.[12] These two supraesophageal reflexes prevent the entry of reflux materials into the larynx, pharynx, and lungs. Defective reflexes might be associated not only with aspiration of gastric contents, but also with reflux-related otolaryngological disorders. To assess the direct effect of cigarette smoking on GPR and GER, we performed ambulatory 24-hour double-probe pH monitoring in cigarette smokers.

PATIENTS AND METHODS

Patients. Fifteen subjects (9 men, 6 women; mean age, 41 years; range, 24 to 61 years) with a history of smoking at least 15 cigarettes per day for more than 5 years were studied.

All subjects underwent 24-hour double-probe pH monitoring in an ambulatory setting. The subjects were asked to continue their normal daily smoking habits during the monitoring.

Eleven subjects presented with otolaryngological complaints (globus, dysphonia); the other 4 were healthy normal subjects (patients 7, 8, 9, and 10).

pH Measurement. Double-probe pH monitoring was performed with 2 monocrystalline antimony pH sensors positioned along a single catheter (diameter, 2.1 mm) with the sensors 15 to 20 cm apart and a silver–silver chloride cutaneous reference electrode (Digitrapper Mark III, Medtronic Synectics, Maastricht, the Netherlands). Both probes (pH sensors) were calibrated simultaneously in buffer solutions pH 7 and pH 1 before monitoring. Under endoscopic view, the proximal probe was placed in the UES in such a way that the probe was just surrounded by the esophageal mucosa. A recent study showed that endoscopic placement of the proximal probe in the UES without manometry is an accurate method.[14] Accordingly, the second probe (distal pH sensor) was positioned 15 or 20 cm distal to the proximal probe, de-

---

From the Department of Otorhinolaryngology–Head and Neck Surgery, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

CORRESPONDENCE — Laki D. Stanojcic, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, Academic Medical Center, University of Amsterdam, PO Box 22700, 1105 DE Amsterdam, the Netherlands.

TABLE 1. NUMBER OF CIGARETTES AND PERCENTAGE OF TIME PH < 4 DURING 24-HOUR PH MONITORING

	No. of Cigarettes		*Proximal Probe (% Time pH < 4)*			*Distal Probe (% Time pH < 4)*		
Patient	*Daily Use*	*During Monitoring*	*Total*	*Upright*	*Supine*	*Total*	*Upright*	*Supine*
1	20	17	0.0	0.0	0.0	1.7	2.4	0.0
2	25	20	0.0	0.0	0.0	1.6	2.1	0.0
3	20	9	0.0	0.0	0.0	2.5	3.5	0.8
4	15	6	0.0	0.0	0.0	0.3	0.5	0.2
5	20	15	0.1	0.1	0.0	0.3	0.5	0.0
6	15	14	0.0	0.0	0.0	0.8	1.2	0.0
7	20	17	0.0	0.0	0.0	3.9	5.7	0.0
8	15	13	0.1	0.1	0.1	2.1	0.6	2.5
9	16	8	0.2	0.3	0.0	1.1	1.7	0.0
10	20	16	0.2	0.4	0.0	0.9	1.5	0.0
11	30	18	1.1	1.6	0.0	4.0	5.6	0.7
12	15	7	0.2	0.3	0.0	2.4	3.4	0.0
13	35	30	0.3	0.3	0.5	12.6	16.4	3.8
14	25	23	2.8	3.9	0.0	5.8	7.9	0.1
15	15	11	0.0	0.0	0.0	8.6	4.2	29.1

pending on the distance from the tip of the nose to the LES. This distance was determined by using the pH transition point (withdrawal technique) in order to achieve a position of the distal probe 5 to 10 cm above the LES.

A pH drop below 4 recorded by the proximal probe was considered to be positive evidence of GPR only if it was preceded by a distal pH drop of similar or larger magnitude.[15]

The subjects were encouraged to eat their regular meals without restrictions and to continue their normal daily and nocturnal routines. In a diary, the subjects reported body position, meals, drinks, and cigarette smoking.

Data Analysis. At the end of the measurement, the pH data from the portable recorder were downloaded into a personal computer for analysis. The percentage of time the pH was below 4 for the total time, time in upright position, and time in supine position, as well as for the smoking-related and non–smoking-related periods was calculated with the EsopHogram software program (Medtronic Synectics).

For the distal probe, the criteria of Richter et al[16] were used to distinguish between physiological and pathological GER. Richter et al defined as normal a pH below 4 less than 5.5% of the total time, less than 8.2% of the time in the upright position, and less than 3% of the time in the supine position.

For the proximal probe placed at the entrance of the esophagus, our normal values for GPR, established recently, were used.[14] We consider percentages of time with a pH below 4 of more than 0.1% for the total time, 0.2% for the time upright, and 0% for the time supine to be pathological.

All pH tracings were reviewed manually in order to assess real reflux episodes at the proximal probe.

Reflux episodes that occurred during the time of smoking and within 8 minutes after ending smoking were considered to be smoking-related. Other reflux episodes that occurred during the time that the subject was awake were considered to be non–smoking-related.

The percentages of time that the pH was below 4 were compared for the smoking-related period and the nonsmoking period by use of Wilcoxon's matched-pairs signed ranks test for nonparametric data (1-tailed).

RESULTS

Seven of the 15 subjects (Nos. 1 through 7) did not have any pathological reflux, according to the abovementioned criteria. Five subjects (Nos. 8 through 12) had pathological values for GPR, 2 subjects (Nos. 13 and 14) had pathological values for GPR as well as GER, and 1 subject (No. 15) had pathological values for GER (Table 1).

The percentages of time the pH was below 4 during the smoking-related period and during the nonsmoking period, measured at the proximal probe and at the distal probe, are shown in Table 2. At the level of the UES, the median percentage of time the pH was below 4 for the smoking period was 0.1%, versus 0% for the nonsmoking period. In the distal esophagus, the median percentage of time the pH

TABLE 2. PERCENTAGE OF TIME PH < 4 WITH NO SMOKING AND DURING SMOKING AT PROXIMAL AND DISTAL PROBES

	Proximal Probe (% Time pH < 4)		*Distal Probe (% Time pH < 4)*	
Patient	*Period Awake and Not Smoking*	*Smoking-Related Period*	*Period Awake and Not Smoking*	*Smoking-Related Period*
1	0	0	2.2	2.6
2	0	0.2	0.9	7.1
3	0	0	2.9	7.7
4	0	0	0.3	1.6
5	0.1	0	0.6	0.1
6	0	0.1	0.7	2.9
7	0	0	5.5	6.2
8	0.1	0	0.8	0
9	0	1.1	1.1	5.8
10	0.3	0.6	0.9	3.5
11	1.1	2.7	4.7	7.2
12	1.4	3.7	4.2	12.6
13	0.9	2.8	3.9	9.8
14	3.4	4.9	6.9	10.0
15	0	0.1	4.0	4.9
Median	0	0.1	2.2	5.8
Range	0-3.4	0-4.9	0.3-6.9	0-12.6

was below 4 for the smoking period was 5.8%, and for the nonsmoking period, 2.2%.

When the percentage of time the pH was below 4 during the smoking period was compared with the percentage of time the pH was below 4 during the nonsmoking period, the difference was statistically significant, proximal, at the level of the UES ($p < .01$), as well as distal, above the LES ($p < .001$).

When the smoking-related periods were excluded from the analysis, the values of 1 subject (No. 14) with pathological GPR and GER turned into normal values for GER. (The percentages of time the pH was below 4 for the total time, time in the upright position, and time in the supine position changed from 5.8%, 7.9%, and 0.1% into 4.5%, 6.8%, and 0.1%.) According to our criteria, the values of 2 subjects (Nos. 9 and 12) with only pathological GPR turned into normal values for GPR. (The percentages of time the pH was below 4 for the total time, time in the upright position, and time in the supine position changed, respectively, from 0.2%, 0.3%, and 0.0% into 0.1%, 0.2%, and 0.0% and from 0.2%, 0.3%, and 0.0% into 0.0%, 0.0%, and 0.0%).

DISCUSSION

In this study, a significant increase of GPR, as well as GER, during smoking was found. Animal studies have shown that minute amounts of gastric fluids are capable of inducing damage to laryngeal structures.[17,18] Furthermore, GPR appears to be associated with various otolaryngological disorders such as hoarseness, chronic throat-clearing, posterior laryngitis, laryngeal granulomas, subglottic stenosis, and carcinomas of the aerodigestive tract.[17-19] Because there is a relationship between smoking and several of these otolaryngological disorders as well, we studied the effect of cigarette smoking on GPR and GER.

To detect GPR and GER, ambulatory 24-hour double-probe pH monitoring was performed. This technique is one of the most reliable methods for detecting both low and high esophageal refluxes of acid gastric juice.[20,21] The advantage of pH monitoring in an ambulatory setting is the achievement of an as-normal-as-possible situation during the monitoring period. Therefore, it is advised for the measurement of GER to minimize restrictions during the 24-hour monitoring period in order to get an as representative a result as possible.[22] Consequently, subjects who smoke should actually continue their normal daily smoking habits during the pH monitoring.

After many attempts to define the most reliable parameter and the best applicable normal values, a consensus was reached that the percentage of time (total, upright, supine) the pH is below 4 is the most useful discriminator between physiological and pathological reflux.[22] The impact of reflux that occurred during the smoking periods, however, was not always detectable when the percentages of time the pH was below 4 (total time, time in upright position, and time in supine position) were calculated by the computer. For example, subject 2 and subject 6 showed some reflux at the proximal probe during the smoking period (Table 2); however, on the overall analysis, these values were reduced to 0.0% (Table 1). Nevertheless, according to our criteria of normal values, the diagnosis would change in 3 subjects (Nos. 9, 12, and 14) if the smoking periods were excluded.

In single-probe pH studies, an increase in reflux episodes distal in the esophagus was also found during smoking,[6,7,11,23] although some investigators could not find a significant increase in the total esophageal acid exposure time.[6,23]

To our knowledge, this is the first study on GER and GPR in subjects who smoke, and our results are in agreement with the finding of Dua et al[12] that smoking may have implications for the development of supraesophageal reflux–related complications (otolaryngological and respiratory).

Cigarette smoking may influence reflux; therefore, smokers with complaints and disorders caused by reflux should strongly be advised to stop smoking.

REFERENCES

1. Dennish GW, Castell DO. Inhibitory effect of smoking on the lower esophageal sphincter. N Engl J Med 1971;284:1136-7.

2. Stanciu C, Bennett JR. Smoking and gastro-oesophageal reflux. Br Med J 1972;3:793-5.

3. Chattopadhyay DK, Greaney MG, Irving TT. Effect of cigarette smoking on the lower oesophageal sphincter. Gut 1977;18:833-5.

4. Kjellin G, Tibbling L. Influence of body position, dry and wet swallows, smoking and alcohol on esophageal acid clearing. Scand J Gastroenterol 1978;13:283-8.

5. Kahrilas PJ, Gupta RR. The effect of cigarette smoking on salivation and esophageal clearance. J Lab Clin Med 1989;114:431-8.

6. Waring JP, Eastwood TF, Austin JM, Sanowski RA. The immediate effects of cessation of cigarette smoking on gastroesophageal reflux. Am J Gastroenterol 1989;84:1076-8.

7. Kahrilas PJ, Gupta RR. Mechanisms of acid reflux associated with cigarette smoking. Gut 1990;31:4-10.

8. Johnson RD, Horowitz M, Maddox AF, Wishart JM, Shearman DJC. Cigarette smoking and rate of gastric emptying: effect on alcohol absorption. BMJ 1991;302:20-3.

9. Scott AM, Kellow JE, Shuter B, Nolan JM, Hoschl R, Jones MP. Effect of cigarette smoking on solid and liquid intragastric distribution and gastric emptying. Gastroenterology 1993;104:410-6.

10. Rahal PS, Wright RA. Transdermal nicotine and gastroesophageal reflux. Am J Gastroenterol 1995;90:919-21.

11. Kadakia SC, Kikendall JW, Maydonovitch C, Johnson LF. Effect of cigarette smoking on gastroesophageal reflux measured by 24-h ambulatory esophageal pH monitoring. Am J Gastroenterol 1995;90:1785-90.

12. Dua K, Bardan E, Ren J, Sui Z, Shaker R. Effect of chronic and acute cigarette smoking on the pharyngo–upper oesophageal sphincter contractile reflex and reflexive pharyngeal swallow. Gut 1998;43:537-41.

13. Boermeester MA, van Sandick JW, van Lanschot JJB, Boeckxstaens GE, Tytgat GNJ, Obertop H. Gastro-oesofageale refluxziekte: pathofysiologie, diagnostiek en medicamenteuze therapie. Ned Tijdschr Geneeskd 1998;142:1306-10.

14. Smit CF, Tan J, Devriese PP, Mathus-Vliegen EMH, Brandsen M, Schouwenburg PF. Ambulatory pH monitoring at the upper esophageal sphincter. Laryngoscope 1998;108:299-302.

15. Katz PO. Ambulatory esophageal and hypopharyngeal pH monitoring in patients with hoarseness. Am J Gastroenterol 1990;85:38-40.

16. Richter JE, DeMeester TR, Wu WC, Sinclair JW, Schan C, Bradley LA. Normal 24-hour esophageal pH values: influence of age and gender [Abstract]. Gastroenterology 1990;98:A122.

17. Koufman JA. The otolaryngologic manifestations of gastroesophageal reflux disease (GERD): a clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury. Laryngoscope 1991;101 (suppl 53).

18. Delahunty JE, Cherry J. Experimentally produced vocal cord granulomas. Laryngoscope 1968;78:1941-7.

19. Toohill RJ, Kuhn JC. Role of refluxed acid in the pathogenesis of laryngeal disorders. Am J Med 1997;103(suppl 5A):100S-106S.

20. Dobhan R, Castell DO. Normal and abnormal proximal esophageal acid exposure: results of ambulatory dual-probe pH monitoring. Am J Gastroenterol 1993;88:25-9.

21. Wiener GJ, Morgan TM, Copper JB, et al. Ambulatory 24-hour esophageal pH monitoring. Reproducibility and variability of pH parameters. Dig Dis Sci 1988;33:1127-33.

22. Kahrilas PJ, Quigley EMM. Clinical esophageal pH recording: a technical review for practice guideline development. Gastroenterology 1996;110:1982-96.

23. Schindlbeck NE, Heinrich C, Dendorfer A, Pace F, Muller-Lissner SA. Influence of smoking and esophageal intubation on esophageal pH-metry. Gastroenterology 1987;92:1994-7.

Ann Otol Rhinol Laryngol 110:2001

BIFID EPIGLOTTIS ASSOCIATED WITH JOUBERT'S SYNDROME

MYUNG-WHUN SUNG, MD
JEONG-WHUN KIM, MD
KWANG HYUN KIM, MD
SEOUL, KOREA

This report presents the case of a 2-year-old boy who had a bifid epiglottis associated with Joubert's syndrome. He had episodic tachypnea and apnea, inspiratory stridor, aspiration, and growth and mental retardation. Direct laryngoscopy demonstrated agenesis of the right half of the epiglottis and hypertrophied mucosa over the arytenoid cartilage. After the hypertrophied mucosa was partially vaporized with a CO_2 laser, the inspiratory stridor soon improved and the aspiration was alleviated. Brain magnetic resonance imaging showed cerebellar vermian agenesis and enlargement of the fourth ventricle.

KEY WORDS — bifid epiglottis, Joubert's syndrome.

INTRODUCTION

Bifid epiglottis is a rare anomaly that causes dyspnea or aspiration and is usually associated with multiple anomalies, including hand or foot polydactyly, cleft lip or palate, hypospadias, imperforate anus, congenital hypopituitarism, and hypothalamic hamartoblastoma. Joubert's syndrome is also rare and shows cerebellar vermian agenesis, episodic tachypnea and apnea in the neonatal period, ocular motor dysfunction, mental retardation, and ataxia. This is the first case report of a patient with a bifid epiglottis associated with Joubert's syndrome.

CASE REPORT

A 2-year-old boy was referred to our clinic. He had been born at 38 weeks to a 27-year-old woman by cesarean section for cephalopelvic disproportion. Soon after birth, episodic tachypnea and apnea were noted. The tachypneic episodes lasted up to 10 minutes. He showed cyanosis during the apneic episodes, lasting approximately 1 minute, which terminated with tactile stimulation. Intubation was not required, despite inspiratory stridor. One month later, his respiration was normal. He had 6 digits on the right foot (Fig 1). Firm, cystic masses were found on the ventral tongue. Because of persistent aspiration with feeding, he failed to gain weight. He had growth retardation and delayed development. Brain magnetic resonance imaging (MRI) demonstrated cerebellar vermian agenesis with enlargement of the fourth ventricle, but other cerebral anomalies, such as hypothalamic hamartoblastoma, were not present (Fig 2). His chromosomes and amino acids were normal. The findings of an endocrinologic evaluation and a renal function test were also normal.

When referred to us, the patient had inspiratory stridor and aspiration. He also had difficulty in swallowing solid food. He showed supraclavicular retraction and pectus excavatum. Laryngeal telescopic examination revealed a rudimentary epiglottis and a prominent arytenoid area with normal vocal cord mobility. He was ophthalmologically normal. The other family members had no significant disorders.

Direct laryngoscopy demonstrated an absence of the right half of the epiglottis and a severe hypoplasia of the left half. The mucosa above the arytenoid cartilage was hypertrophied. There was no associated anomaly of the hypopharynx or larynx (Fig 3). The hypertrophied mucosa over the arytenoids was

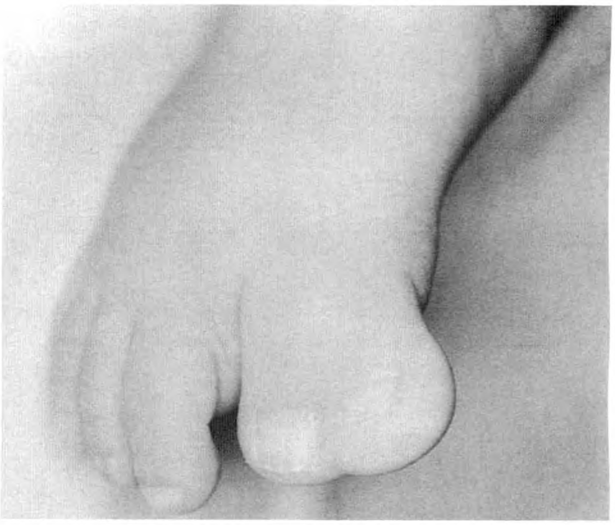


Fig 1. Polydactyly of right foot. Six well-formed digits are shown.

From the Department of Otorhinolaryngology–Head and Neck Surgery, Seoul National University College of Medicine, and the Clinical Research Institute, Seoul National University Hospital, Seoul, Korea. This study was supported in part by the 1999 BK21 Project for Medicine, Dentistry and Pharmacy.

CORRESPONDENCE — Myung-Whun Sung, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, Seoul National University College of Medicine, 28 Yongon-Dong, Chongno-Gu, Seoul, 110-744, Korea.

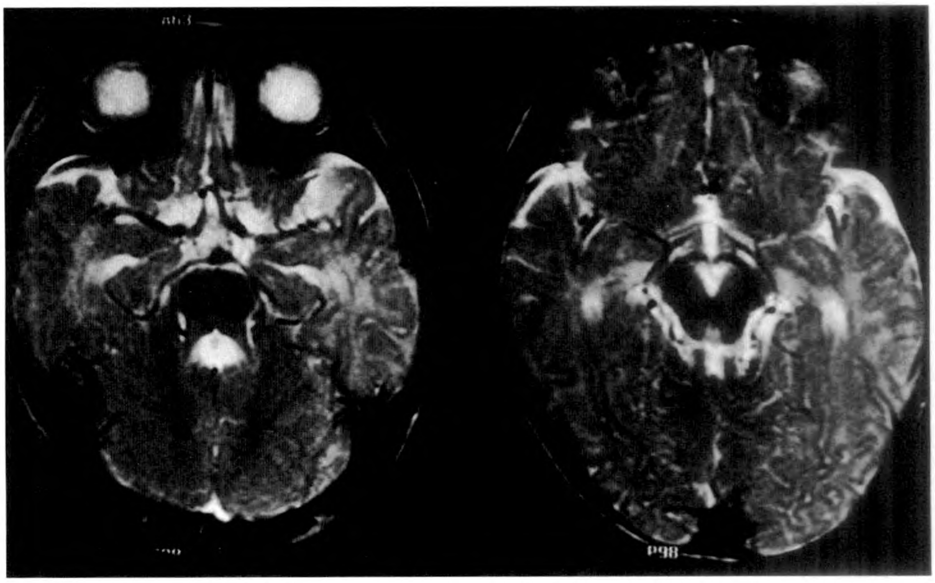


Fig 2. Brain magnetic resonance imaging shows agenesis of cerebellar vermis and enlargement of fourth ventricle.

partially vaporized with a CO_2 laser.

The patient's inspiratory stridor improved from the first postoperative day. Aspiration was more prominent than in the preoperative state for 2 weeks. During the follow-up period, the aspiration was alleviated and the patient became able to swallow solid foods. Because of ataxia, he was placed on rehabilitation therapy. Excisional biopsy of the tongue mass (lipoma) and amputation of the additional digit were performed 6 months later.

DISCUSSION

A true bifid epiglottis has a midline cleft along its entire length, and the epiglottis is divided into 2 cartilaginous halves.[1] Only 5 of the 17 cases that Montreuil[2] reviewed had true bifid epiglottis. Embryologically, it is not clear whether the epiglottis is of central or bilateral origin.[1,3,4] A hypobranchial eminence, the epiglottic precursor, is formed just inferior to the tongue and superior to the epiglottis at day 32. Its anterior portion contributes to the development of the tongue, and its posterior portion to the formation of the epiglottis.[3] It is controversial whether the hypobranchial eminence is of bilateral origin or not, and whether it is the sole anlage of the epiglottis. According to the theory of bilateral origin, the hypobranchial eminence arises from the third and fourth branchial arches.[1,3] In our case, the division of the epiglottis, with the right half absent and the left hypoplastic, may support the theory of bilateral development.

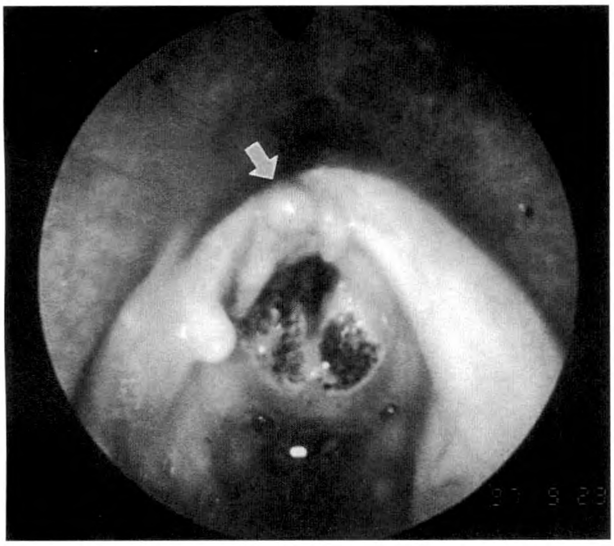


Fig 3. Photograph shows hypoplasia of left half (arrow) and aplasia of right half of bifid epiglottis. Hypertrophied arytenoid mucosa was vaporized with CO_2 laser. Glottis is rotated slightly clockwise from midline because of rigid laryngoscope.

Patients with bifid epiglottis usually have dyspnea with inspiratory stridor and/or aspiration with feeding just after birth. It is easily diagnosed by direct or indirect laryngoscopy. Other probable anomalies associated with bifid epiglottis should be sought, including hand or foot polydactyly, cleft lip or palate, hypospadias, imperforate anus, congenital hypopituitarism, and hypothalamic hamartoblastoma. McClay et al,[5] in 1997, reported bifid epiglottis in 11 members of a family spanning 3 generations. Only 2 patients had hand polydactyly, and 9 had other anomalies in addition. Direct laryngoscopy is recommended to determine whether other laryngeal abnormalities are associated.

In patients presenting with tolerable aspiration or dyspnea, a regular follow-up will suffice after verification of the laryngeal anomaly with direct laryngoscopy under general anesthesia.[3] Montreuil[2] amputated the epiglottis in his case, because it collapsed into the laryngeal inlet on inspiration to cause inspiratory stridor and dyspnea. The dyspnea resolved completely after surgery, but there was no mention of aspiration in their report. In the review of McClay et al,[5] 3 patients underwent amputation of all or part of the epiglottis. Dyspnea resolved, and aspiration did not develop.

Cerebellar vermian agenesis is found in Joubert's syndrome, as well as Dandy-Walker malformation. However, the latter differs from the former in that it involves hydrocephalus, heterotopia of the cerebellar and cerebral cortex, anomalies of medullary centers with a widespread distribution, and cystic dilatation of the fourth ventricle.[6,7] Among various anomalies occurring in the posterior cranial fossa, Joubert's syndrome is the only one with respiratory abnormality.[8] The tachypneic episodes last up to 20 minutes, with 80 to 100 respirations per minute, and apneic episodes last up to 2 minutes.[6] This episodic dyspnea usually resolves within 1 month. Abnormal ocular movements vary from nystagmus to impaired smooth pursuit, or to a saccade palsy.[6] Mental retardation is thought to be caused by diffuse functional disorders of the cerebellum and brain stem. Polydactyly, cleft palate, cystic kidney disease, and retinal dystrophy can be associated,[8] but laryngeal anomalies have not yet been reported. As its manifestations include cleft epiglottis and polydactyly, Pallister-Hall syndrome should also be differentiated.[9,10] Pallister-Hall syndrome is characterized by hypothalamic hamartoblastoma, which can also be present in Joubert's syndrome. However, our patient did not have hypothalamic hamartoblastoma and had cerebellar vermian agenesis, which is a typical finding of Joubert's syndrome. From the standpoint of treatment, there is no effective therapy for Joubert's syndrome, and the 5-year survival rate is 50%. The most common causes of death are severe feeding difficulty and respiratory infection.[8]

This is the first case reported of both bifid epiglottis and Joubert's syndrome. The possibility of a congenital laryngeal anomaly should be considered in all infants with dyspnea and aspiration. In addition to the airway, an examination of the whole body is required to find another associated anomalies. Physicians should think of abnormalities in the central nervous system when patients have abnormal respiration, muscular hypotonia, or abnormal ocular movements, and physicians also should think of structural abnormalities of the aerodigestive tract when anatomic abnormalities of the cranial nervous system exist with aerodigestive tract symptoms such as stridor or chronic aspiration.

REFERENCES

1. Goldenberg JD, Holinger LD, Bressler FJ, Hutchinson LR. Bifid epiglottis. Ann Otol Rhinol Laryngol 1996;105:155-7.

2. Montreuil F. Bifid epiglottis, report of a case. Laryngoscope 1949;59:194-9.

3. DelMonico ML, Haar JG. Bifid epiglottis: report of a case. Arch Otolaryngol 1972;96:178-81.

4. Hast MH. The developmental anatomy of the larynx. Otolaryngol Clin North Am 1970;3:413-38.

5. McClay JE, Wiatrak B, Proud VK. Bifid epiglottis and polydactyly: a new genetic syndrome. Otolaryngol Head Neck Surg 1997;116:129-33.

6. Egger J, Bellman MH, Ross EM, Baraitser M. Joubert-Boltshauser syndrome with polydactyly in siblings. J Neurol Neurosurg Psychiatry 1982;45:737-9.

7. Kendall B, Kingsley D, Lambert SR, Taylor D, Finn P. Joubert syndrome: a clinico-radiological study. Neuroradiology 1990;31:502-6.

8. Suzuki T, Hakozaki M, Kubo N, Kuroda K, Ogawa A. A case of cranial meningocele associated with Joubert syndrome. Childs Nerv Syst 1996;12:280-2.

9. Verloes A, David A, Ngo L, Bottani A. Stringent delineation of Pallister-Hall syndrome in two long surviving patients: importance of radiological anomalies of the hands. J Med Genet 1995;32:605-11.

10. Squires LA, Constantini S, Miller DC, Wisoff JH. Hypothalamic hamartoma and the Pallister-Hall syndrome. Pediatr Neurosurg 1995;22:303-8.

Ann Otol Rhinol Laryngol 110:2001

EPIGLOTTIC TUBERCULOSIS: DIFFERENTIAL DIAGNOSIS AND TREATMENT

CASE REPORT AND REVIEW OF THE LITERATURE

BERNHARD RICHTER, MD
FREIBURG, GERMANY
GABRIELE KÖHLER, MD
FREIBURG, GERMANY
MILO FRADIS, MD
HAIFA, ISRAEL
GERD JÜRGEN RIDDER, MD
FREIBURG, GERMANY

A case of a 40-year-old man with tuberculous involvement of the epiglottis suffering from unsuspected pulmonary tuberculosis is described. The laryngeal lesions were primarily considered to be highly suspicious for a neoplastic process rather than an infectious one. After diagnosis, the patient was treated according a standard protocol and followed up for a period of 2 years. He is still free of disease. The clinical presentation, diagnosis, pathological findings, and therapy of the condition are described. The differential diagnosis and management of epiglottic tuberculosis are reviewed and discussed. Even though these cases are rare, otorhinolaryngologists should keep in mind the possibility of tuberculosis in the differential diagnosis of laryngeal tumors, as the incidence of tuberculosis in developed countries is steadily increasing.

KEY WORDS — epiglottis, larynx, tuberculosis.

INTRODUCTION

Tuberculosis of the larynx is quite rare and is seldom considered in the differential diagnosis of laryngeal diseases.[1,2] Awareness of the disease is important, as it is frequently misdiagnosed as a malignant laryngeal tumor or granulomatous disease.[1,3-5] Tuberculosis shows a rising incidence worldwide,[6] including the developed countries, and the World Health Organization has declared tuberculosis a global emergency.[7]

In the field of otorhinolaryngology, the vocal folds are the site most commonly affected, closely followed by the ventricular band.[8] The other sites affected in approximately 10% to 15% of cases are the aryepiglottic folds, arytenoids, posterior commissure, subglottis, and epiglottis. The anterior half of the larynx is affected twice as frequently as the posterior half.

Isolated epiglottic tuberculosis, however, is uncommon and has rarely been described.[3,9-11] Bobrov[3] reported 3 cases of tuberculous epiglottitis, 1 of them mimicking epiglottic carcinoma.

In this paper we describe a case of epiglottic involvement that was thought to be an epiglottic malignancy in a patient suffering from unsuspected pulmonary tuberculosis.

CASE REPORT

A 40-year-old man was referred to the otolaryngology department of the University Hospital Freiburg, Germany, to rule out carcinoma of the larynx because of a 3-month history of difficulty in swallowing, hoarseness, and chronic cough. He had been treated by his attending physician with antibiotics without any improvement. He was a heavy cigarette smoker (about 30 pack-years). His medical history was remarkable for intravenous drug abuse (heroin) 10 years earlier. Indirect laryngoscopy showed a severely swollen epiglottis with superficial granulating and partially necrotic mucosa. The vocal folds appeared not to be affected. They showed normal mobility. Small cervical lymph nodes in the jugular digastric angle were palpable. The remaining findings on the otolaryngologic and physical examinations were normal. Chest radiography showed confluent infiltration in the apices of both lungs and thickening of the pleura in the left lung area (Fig 1). In spite of the laryngeal appearance that suggested a carcinoma, the chest radiograph findings raised the possibility of tuberculosis. A Mantoux test was performed and was found to be highly positive.

Human immunodeficiency virus (HIV) serology by enzyme-linked immunosorbent assay was negative. Serologic studies of other specific granulomatous diseases, including syphilis, cat-scratch disease, and fungal infections (histoplasmosis, blastomycosis, coccidioidomycosis), were negative. There was

---

From the Departments of Otorhinolaryngology–Head and Neck Surgery (Richter, Ridder) and Pathology (Köhler), University Clinic Freiburg, Freiburg, Germany, and the Department of Otolaryngology–Head and Neck Surgery, Bnai Zion Medical Center, Faculty of Medicine Technion, Haifa, Israel (Fradis).

CORRESPONDENCE — Bernhard Richter, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, University Clinic Freiburg, Killianstrasse 5, D-79106 Freiburg im Breisgau, Germany.

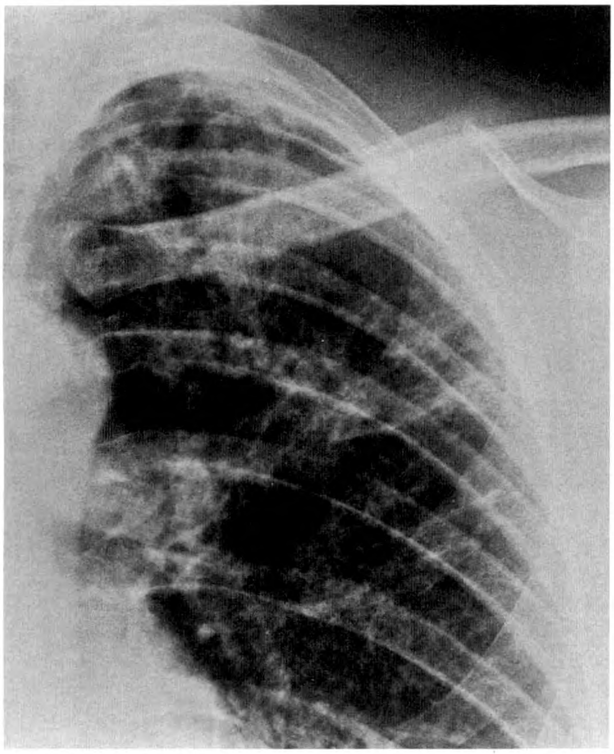


Fig 1. Chest radiograph of left lung shows confluent infiltration in apex and thickening of pleura.

no clinical evidence of Wegener's granulomatosis or sarcoidosis. Acid-fast bacilli seen on a Ziehl-Neelsen stain were found in the sputum and gastric fluid. Under general anesthesia, with the necessary precautions taken in order to minimize the risk of infection, direct laryngoscopy was performed (Fig 2); one biopsy specimen was sent for bacteriologic evaluation and was found to be positive for acid-fast bacteria. Histologic examination of a second biopsy specimen revealed an epithelioid giant cell granulomatous epiglottitis with microfocal caseous necrosis surrounded by lymphocytes and plasma cells (Fig 3). On the basis of the bacteriologic, radiologic, and histologic findings, the diagnosis of pulmonary tuberculosis with epiglottic involvement was established. For further treatment, the patient was transferred to the pneumology department and was started on antimycobacterial therapy (isoniazid, rifampicin, ethambutol hydrochloride, and pyrazinamide). Within 2 weeks, the patient's symptoms had markedly improved. After 6 months of treatment, the appearance of the larynx had returned to normal. All complaints of hoarseness and swallowing difficulties had disappeared. After a follow-up period of 2 years, the patient is still free of pulmonary and laryngeal disease.

DISCUSSION

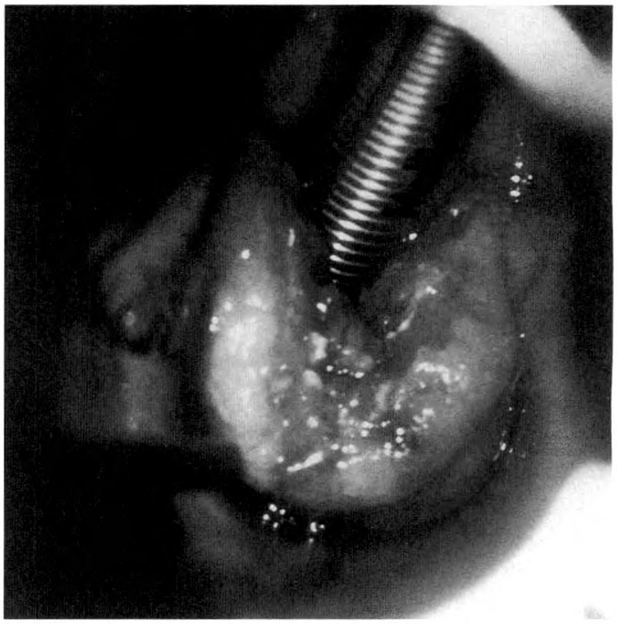


Fig 2. Direct laryngoscopy under general anesthesia shows severely swollen epiglottis with superficially granulating, partially necrotic mucosa.

The clinical presentations of patients with tuberculosis and carcinoma of the larynx may be similar, including hoarseness, dysphagia, dyspnea, cough, and sore throat. Patients often report a history of weight loss and tobacco or alcohol abuse.[12] Under laryngoscopy, tuberculous laryngitis can be indistinguishable from carcinoma or chronic laryngitis.

In our case, difficulties in swallowing and hoarseness, in addition to a severely swollen epiglottis with granulating and necrotic mucosa, led the attending physician to suspect laryngeal carcinoma. However, a chest radiograph and a Mantoux test made tuberculosis a likely cause of the lesion of the larynx. The latter diagnosis was confirmed by histologic examination.

Nedwicki[13] described 2 patients who underwent direct laryngoscopy and biopsy for presumed carcinoma of the larynx and were found to have tuberculosis.

Bull[14] reported 7 patients with laryngeal tuberculosis masquerading as carcinomas, and later, Bobrov[3] reported 1 more case of misdiagnosed laryngeal tuberculosis. Smallman et al[15] reviewed 6 cases of laryngeal tuberculosis in a 10-year period, stressing the point that the symptoms and signs often mimic carcinoma, chronic laryngitis, or other granulomatous disease of the larynx.

In the past, epiglottic involvement was rarely seen, but in the last decade there has been a changing pattern of laryngeal tuberculosis, as supported by the finding of Singh et al[16] in 1996 that 1 of their 8 non–HIV-positive patients had the lesion localized at the epiglottis. Kandiloros et al[17] reported 9 cases of la-

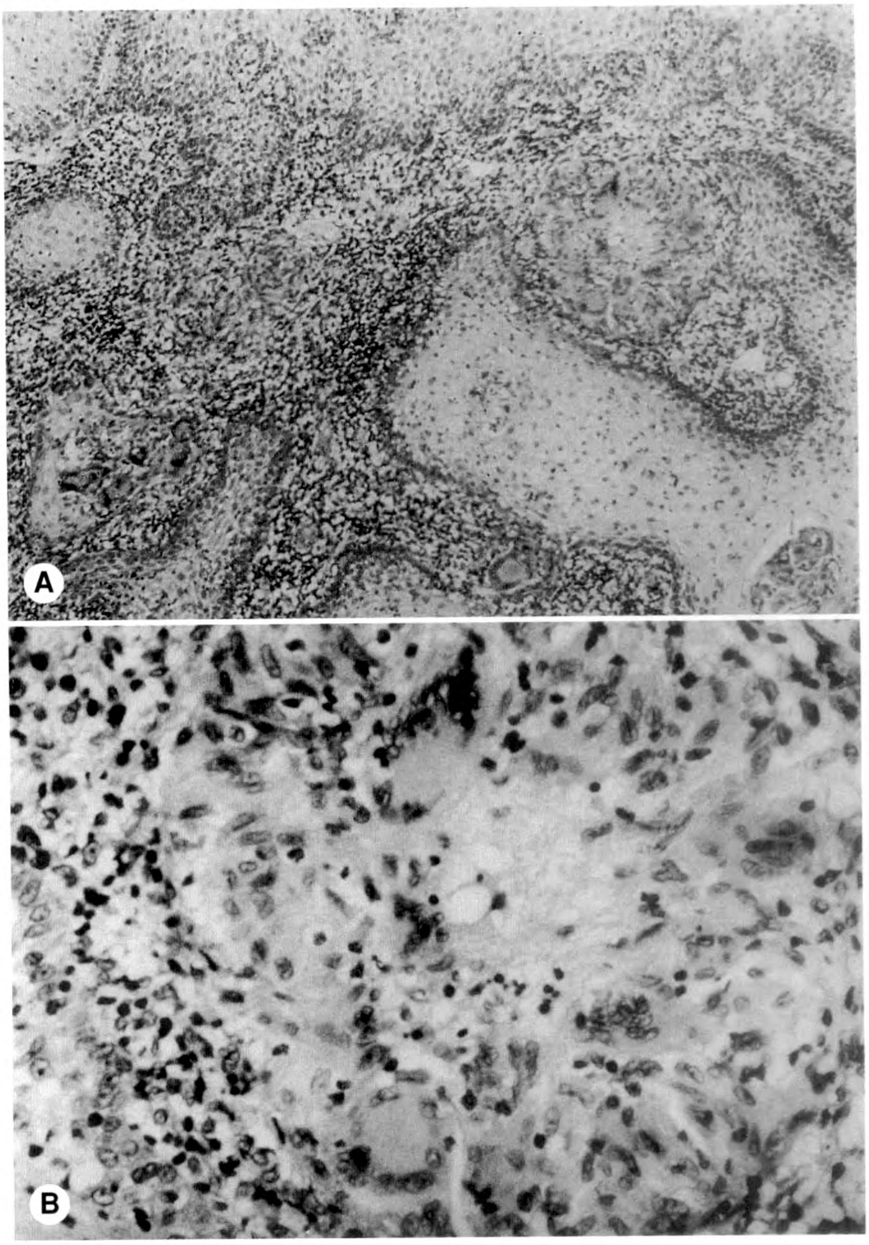


Fig 3. Histology of epiglottic biopsy. A) Epiglottitis with microfocal caseous necrosis (H & E, original ×100). B) Epithelioid giant cell epiglottitis with microfocal caseous necrosis surrounded by lymphocytes and plasma cells (H & E, original ×400).

ryngeal tuberculosis, and found the lesion at the epiglottis in 2 cases. Thaller et al[18] reviewed 15 patients with laryngeal tuberculosis, finding only 1 patient with isolated epiglottic involvement, and 1 patient with involvement of the epiglottis and aryepiglottic folds. In a series of 31 cases of tuberculous laryngitis, Agarwal and Bais[19] described only 1 patient in whom the epiglottis represented the only site of laryngeal lesion.

Couldery[1] in 1990 stressed the necessity of considering the possibility of tuberculosis in the differential diagnosis of laryngeal lesions. Gallas et al[20] found 738 cases of laryngeal tuberculosis when reviewing the English- and French-language literature from 1965 to 1991 and added 4 personal cases, 2 of them in HIV-positive patients. Of those 738 cases, 60.5% were located in only one region of the larynx, most frequently at the vocal cords. In 39.5%, a wider spread was found, and in 28.4%, the epiglottic region was also involved.[20] Tu et al[21] reviewed 46 patients with laryngeal tuberculosis and stated that the epiglottis was affected in 39% of the cases in addition to other parts of the larynx. Rupa and Bhanu[22] found the anterior part of the true vocal cords to be the chief area affected. The subglottis is a site rarely involved in laryngeal tuberculosis.[23]

The pathogenesis of laryngeal tuberculosis is either primary or secondary.[24] Primary infection of the larynx occurs when there is absence of pulmonary disease. The bacillus is transmitted by aerosolized particles and infects the mucosa of the larynx. Secondary infection of the larynx may occur in 1 of 3 ways. First, the patient may have advanced pulmonary disease, usually cavitary, the larynx being inoculated by infected sputum. Second, hematogenous dissemination may seed to the larynx. Third, lymphatic drainage from affected tissue may seed to the larynx. The last two situations seem to be very rare. When the suspicion arises, a chest radiograph and acid-fast smears for tuberculosis should be obtained before a biopsy is undertaken. Tuberculosis of the epiglottis is usually subsequent to cavitary pulmonary tuberculosis.[5,25] Rarely, it is associated with miliary tuberculosis. Soda et al[8] postulated that laryngeal involvement is more commonly caused by hematogenous or lymphatic spread of the bacillus. The most probable reasons for the resurgence of tuberculosis are the wide epidemic of HIV, bad living and nutritional conditions, and mycobacterial strains with rising resistance to most available chemotherapeutic agents.[7,26]

Cervical and submandibular lymphadenopathy are not helpful in the differential diagnosis, although reports in the literature suggest that cervical adenopathy is more frequently encountered with carcinoma.[5] Tuberculous cervical lymphadenitis develops in approximately 5% of all patients with tuberculosis.[27]

Edema of the epiglottis may become quite marked as the disease progresses and is probably secondary to lymphatic obstruction by the granulomas.[28] In the majority of cases, no deep submucosal infiltration of the epiglottic and paralaryngeal fat spaces is seen when there is involvement of the laryngeal mucosa.[29]

The differential diagnostic considerations for granulomatous lesions of the epiglottis include tuberculosis, syphilis, sarcoidosis, Wegener's granulomatosis, cat-scratch disease, fungal infections (histoplasmosis, blastomycosis, coccidioidomycosis), and neoplastic lesions.[18] Other chronic laryngeal infections are cryptococcosis, actinomycosis, candidiasis, and leprosy. Systemic lupus erythematosus, rheumatoid arthritis, relapsing polychondritis, and amyloidosis are systemic diseases that can also affect the larynx and should be considered in the differential diagnosis.

Treatment by corticosteroids for Wegener's granulomatosis could influence the patient's condition detrimentally if misdiagnosed.[1] Therefore, a history, a Mantoux test, chest radiography, acid-fast sputum smears, and bacteriologic culture of a biopsy specimen are mandatory when a suspicion of tuberculosis arises. Fungi, syphilis, and sarcoidosis could present with similar clinical and laryngoscopic findings and should be correctly diagnosed, but the major problem is the differential diagnosis of carcinoma of the larynx.[4,5,13]

There are reports that the incidence of tuberculosis of the upper respiratory tract may be increasing.[12,17,30] Thus, a specimen should be sent for bacteriologic evaluation of suspicious lesions. Biopsy is the cornerstone of an appropriate diagnosis, because both tuberculosis and malignancy may coexist in the same patient.[6,17,31,32] Therefore, the diagnostic challenge is, first, to rule out cancer, and then to establish the correct diagnosis.

The response of epiglottic tuberculosis to antituberculosis therapy is excellent, with most lesions disappearing over a 2-month period, as in the case of our patient.[14,18]

Even in cases of isolated epiglottic involvement, as presented here, otorhinolaryngologists should include a tuberculotic lesion in differential diagnostic considerations. It should always be suspected in cases presenting with hoarseness and painful dysphagia, in order to isolate and treat these cases at an early stage. Otorhinolaryngologists should be aware of the increasing incidence of tuberculosis of the larynx and the changing patterns of the disease, not only in the developing countries, but also in the developed ones.

REFERENCES

1. Couldery AD. Tuberculosis of the upper respiratory tract misdiagnosed as Wegener's granulomatosis. An important distinction. J Laryngol Otol 1990;104:255-8.
2. Konishi K, Yamane H, Iguchi H, et al. Study of tuberculosis in the field of otorhinolaryngology in the past 10 years. Acta Otolaryngol Suppl (Stockh) 1998(suppl 538):244-9.
3. Bobrov VM. Tuberculous forms of epiglottitis. Vestn Otorinolaringol 1991;4:42-4.
4. Lightfoot SA. Laryngeal tuberculosis masquerading as carcinoma. J Am Board Fam Pract 1997;10:374-6.
5. Naraqi S, Raiser MW, Richards NM, Andersen BR. Tuberculosis of the larynx masquerading as carcinoma. Ann Otol Rhinol Laryngol 1976;85:547-8.
6. Millard FJ. The rising incidence of tuberculosis. J R Soc Med 1996;89:497-500.
7. Nakajima H. Tuberculosis: a global emergency. World Health Forum 1993;14:438.
8. Soda A, Rubio H, Salazar M, Ganem J, Berlanga D, Sanchez A. Tuberculosis of the larynx: clinical aspects in 19 patients. Laryngoscope 1989;99:1147-50.
9. Ruiz Franco MF, Gil Velez M, Hellin Meseguer D, Paya Perez L, Vera Quiles F. A case of tuberculous epiglottitis: an unusual form of presentation. Acta Otorrinolaringol Esp 1997; 48:501-3.
10. Chiang CY, Liu JJ, Chau WY, Hwang TZ. Laryngeal tuberculosis simulating epiglottitis. A case report. Kao Hsiung I Hsueh Ko Hsueh Tsa Chih 1989;5:122-5.
11. Khan I. Tuberculous granuloma of the epiglottis. J Lar-

yngol Otol 1983;97:969-71.

12. Ramadan HH, Tarazi AE, Baroudy FM. Laryngeal tuberculosis: presentation of 16 cases and review of the literature. J Otolaryngol 1993;22:39-41.

13. Nedwicki EG. Laryngeal tuberculosis simulating carcinoma of the larynx. Med Times 1970;98:181-7.

14. Bull TR. Tuberculosis of the larynx. Br Med J 1966;2: 991-2.

15. Smallman LA, Clark DR, Raine CH, Proops DW, Shenoi PM. The presentation of laryngeal tuberculosis. Clin Otolaryngol 1987;12:221-5.

16. Singh B, Balwally AN, Nash M, Har-El G, Lucente FE. Laryngeal tuberculosis in HIV-infected patients: a difficult diagnosis. Laryngoscope 1996;106:1238-40.

17. Kandiloros DC, Nikolopoulos TP, Ferekidis EA, et al. Laryngeal tuberculosis at the end of the 20th century. J Laryngol Otol 1997;111:619-21.

18. Thaller SR, Gross JR, Pilch BZ, Goodman ML. Laryngeal tuberculosis as manifested in the decades 1963-1983. Laryngoscope 1987;97:848-50.

19. Agarwal P, Bais AS. A clinical and videostroboscopic evaluation of laryngeal tuberculosis. J Laryngol Otol 1998; 112:45-8.

20. Gallas D, Coste A, Bedbeder P, Peynegre R. Aspects actuels de la tuberculose laryngée. A propos de quatre cas et revue de la littérature. Ann Otolaryngol Chir Cervicofac 1994; 111:201-7.

21. Tu HY, Li HY, Huang TS. Laryngeal tuberculosis: a series of 46 patients. Chang Keng I Hsueh 1997;20:94-9.

22. Rupa V, Bhanu TS. Laryngeal tuberculosis in the eighties. An Indian experience. J Laryngol Otol 1989;103:864-8.

23. Almeyda J, Tolley NS, Ghufoor K, Mochoulis G. Subglottic stenosis secondary to tuberculosis. Int J Clin Pract 1997; 51:402-3.

24. Ramadan HH, Wax MK. Laryngeal tuberculosis. A cause of stridor in children. Arch Otolaryngol Head Neck Surg 1995; 121:109-12.

25. Galietti F, Giorgis GE, Gandolfi G, et al. Examination of 41 cases of laryngeal tuberculosis observed between 1975-1985. Eur Respir J 1989;2:731-2.

26. Cleary KR, Batsakis JG. Mycobacterial disease of the head and neck: current perspective. Ann Otol Rhinol Laryngol 1995;104:830-3.

27. Shikhani AH, Hadi UM, Mufarrij AA, Zaytoun GM. Mycobacterial cervical lymphadenitis. Ear Nose Throat J 1989;68: 660-72.

28. Goyal A, Nagarkar NM, Uppal KS, Mohan H, Dass A. Tuberculosis of the pyriform fossa. A rare entity. J Laryngol Otol 1998;112:782-3.

29. Moon WK, Han MH, Chang KH, et al. Laryngeal tuberculosis: CT findings. AJR Am J Roentgenol 1996;166:445-9.

30. Williams RG, Douglas-Jones T. Mycobacterium marches back. J Laryngol Otol 1995;109:5-13.

31. Delap TG, Lavy JA, Alusi G, Quiney RE. Tuberculosis presenting as a laryngeal tumour. J Infect 1997;34:139-41.

32. Vidal R, Mayordomo C, Miravitlles M, Marti S, Torrella M, Lorente J. Pulmonary and laryngeal tuberculosis. Study of 26 patients. Rev Clin Esp 1996;196:378-80.

≈

SIXTH ASIAN RESEARCH SYMPOSIUM IN RHINOLOGY

The Sixth Asian Research Symposium in Rhinology will be held March 10-11, 2001, in Hong Kong, China. For information, contact 6th ARSR Secretariat, Department of Surgery, University of Hong Kong Medical Centre, Queen Mary Hospital, Pokfulam, Hong Kong SAR, China; telephone: (852) 2818 0232 or (852) 2855 4235; fax: (852) 2818 1186; e-mail: mededcon@hku.hk.

Ann Otol Rhinol Laryngol 110:2001

IMAGING CASE STUDY OF THE MONTH

CHONDROSARCOMA OF THE NASAL SEPTUM

PASQUALE BLOTTA, MD
FRANCESCO CARINCI, MD
LUIGI CAVAZZINI, MD
STEFANO PELUCCHI, MD
ALESSANDRO MARTINI, MD
FERRARA, ITALY

INTRODUCTION

Chondrosarcoma arising in the nasal septum is an uncommon lesion, with fewer than 40 cases reported in the English-language literature.[1-16] Moreover, in only 1 of these reports is the role of modern imaging techniques stressed.[14] In fact, both computed tomography (CT) and magnetic resonance imaging (MRI) are extremely useful, not only in defining the location, dimensions, and involvement of surrounding structures, but also in providing diagnostic clues — all information of critical importance when planning invasive diagnostic or therapeutic procedures. The characteristic ring-forming calcifications seen on CT scans can point to the correct diagnosis and are related to the pattern of calcification inside the tumor.[14] On the other hand, MRI studies may allow an accurate definition of tumor extension, which is of critical importance, because radical resection is the treatment goal.

CASE REPORT

A 27-year-old woman presented with a 1-year history of bilateral nasal obstruction associated with snoring and rhinorrhea. Her medical history was unremarkable.

On physical examination, both nasal cavities were seen to be filled by a tumor arising from the nasal septum. The overlying mucosa was pale, with no erosion. The mass was hard and painless. Neither regional lymphadenopathy nor additional head and neck lesions were observed. The findings of hematologic and biochemical profiles were normal, as were those of chest radiographs and neck sonography.

Computed tomographic scanning showed a tumor arising from the middle portion of the nasal septum, filling both nasal fossae (Fig 1). The mass was irregular, with ring-forming spotty calcifications, and had a maximum diameter of 6 cm. The lateral walls of both nasal cavities were displaced laterally, and the remaining maxillary sinuses were opaque. The lower ethmoidal cells were destroyed, and small bony erosions were present on both orbital floors. The sphenoid and upper ethmoid structures were uninvolved.

On MRI, the tumor had a low-intensity signal on T1-weighted images, and a high-intensity signal on T2-weighted images. Irregular enhancement was seen on T1-weighted images after gadolinium injection (Fig 2A). The MRI studies provided a better definition of the relationship between the tumor and the inferior rectus muscles, which were displaced but not infiltrated (Fig 2B).

A biopsy under general anesthesia was performed; the intraoperative diagnosis was chondroma, and an excision through a midfacial degloving approach was decided on. The final histologic examination, however, confirmed the clinical suspicion, with a pathology report of a grade 2 chondrosarcoma with negative margins (Fig 3). The patient was discharged from the hospital 1 week after surgery, and at the $1^{1}/_{2}$-year follow-up, she was still alive with no evidence of recurrent disease.

DISCUSSION

Chondrosarcoma of the nasal septum has a specific pattern of presentation, growth, and invasiveness. Nasal obstruction and headache[14] are the most frequent presenting symptoms, whereas cranial nerve involvement and diplopia indicate a delay in diagnosis.[1-3] Intermittent nose bleeding may infrequently occur in the early stages.[10] If associated with hemangiomas and multiple enchondromas, the tumor can be part of Maffucci's syndrome.[12]

There are no reports of neck node involvement or distant metastases, and fatal events are due to intracranial extension of the disease.[2,5,9] Recurrences may occur even 7 years after diagnosis[9]; thus, lifelong follow-up may be warranted.

From the Departments of Otolaryngology (Blotta, Carinci, Pelucchi, Martini) and Pathology (Cavazzini), University of Ferrara, Ferrara, Italy. Dr Blotta is currently in the Department of Otolaryngology, Civil Hospital, Crema, Italy.

CORRESPONDENCE — Alessandro Martini, MD, ENT Clinic, University of Ferrara, Corso Giovecca 203, 44100 Ferrara, Italy.

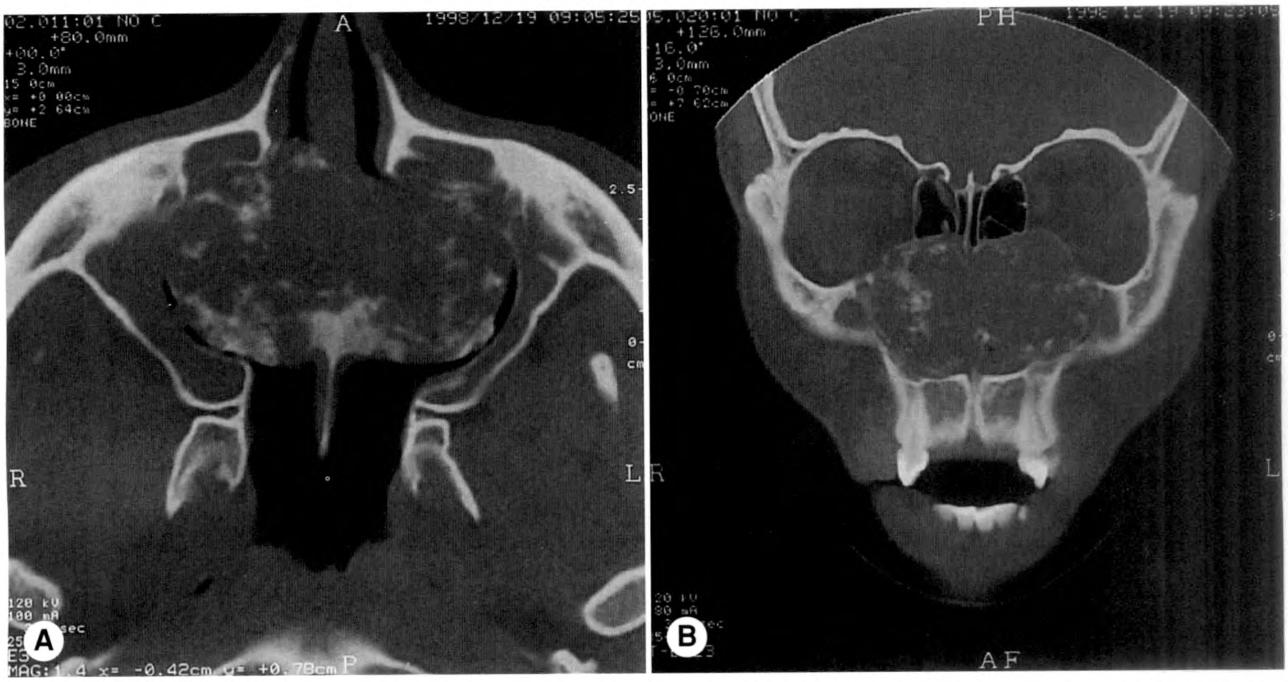


Fig 1. Preoperative computed tomograms. A) Axial scan shows tumor filling both nasal cavities and maxillary sinuses. B) Coronal scan shows relationship between tumor and orbital cones. Note spotty, ringlike calcifications inside mass.

Because local control represents the most important goal when dealing with this tumor, radical surgery is the primary treatment option. In cases of inoperable recurrence, residual disease, or inoperable lesions at onset, radiotherapy[14,16] and radiochemotherapy[2,8,11,13] have been used. Because of the limited number of cases, however, no firm conclusion on the efficacy of these adjuvant treatment methods can be drawn.

Histologically, chondrosarcomas are divided into 3 grades (from 1 to 3) on the basis of cellularity, nuclear size and atypia, and mitotic activity.[13,14] Besides resectability, histologic grading is one of the most important predictors of outcome.

Today, conventional radiology has limited diagnostic value, even if some "stigmata" can be seen on x-ray: Gray and Leonard[3] first reported scattered

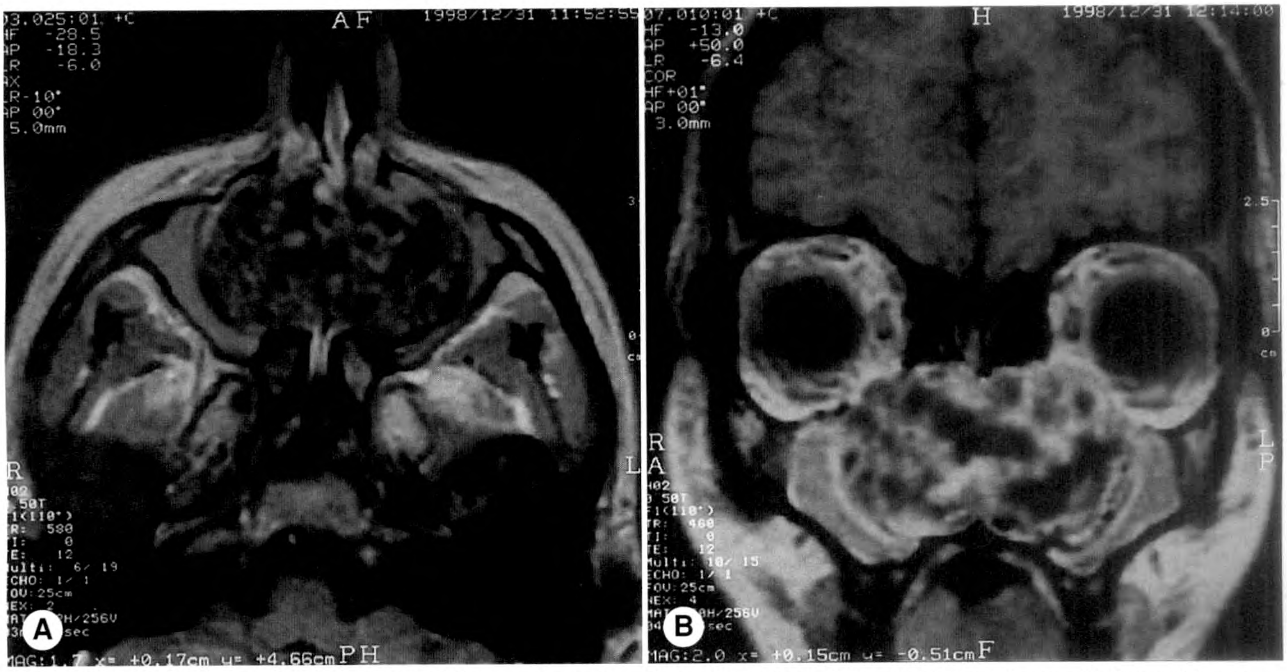


Fig 2. Magnetic resonance images. A) Axial image shows irregular enhancement after gadolinium infusion. B) Coronal image shows relationship between mass and inferior rectus muscles.

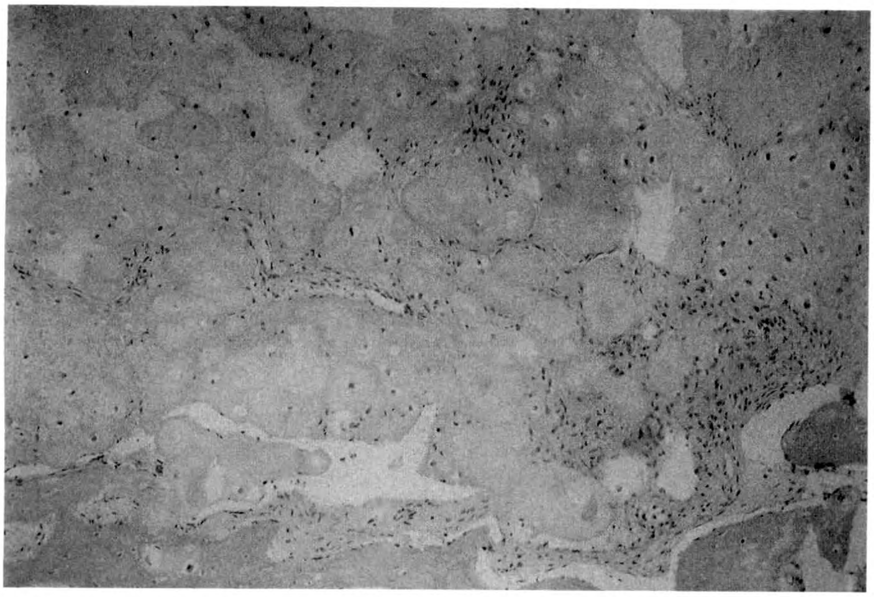


Fig 3. Microphotograph shows grade 2 chondrosarcoma characterized by chondroid stroma, mild variation in size of chondrocytes with enlarged nuclei, rare mitoses, and binucleated cells (H & E, original ×40).

punctate calcifications throughout the tumor as a characteristic sign. In the early 1980s, some authors stressed that CT did not provide reliable information on tumor extension,[4,5] but in the 1990s, CT and MRI became the preferred tools for defining primary tumor site and local extension.[11,15,16] Rassekh et al[14] have correlated imaging and the clinicopathological picture for diagnostic purposes: a CT scan that shows a hypodense matrix with scattered calcifications, areas of ring-forming calcifications, and bony erosions (typical of an expanding mass) is highly suggestive of this lesion. Indeed, ringlike calcifications surrounding tumor lobules can be observed microscopically and account for the spotty images seen on CT.[14] On MRI, a low-intensity signal on T1-weighted images and a high-intensity signal on T2-weighted images are seen. After gadolinium administration, an irregular enhancement is observed on T1-weighted images. Magnetic resonance imaging is extremely accurate in defining the tumor extent and its relationship to surrounding structures (such as the extrinsic muscles of the orbit, in our case).

The radiologic differential diagnosis includes osteosarcoma and chondroma. In osteosarcoma, the calcifications seen on CT scans tend to be linear, rather than spotty, whereas in chondromas, they are usually located around the tumor, in areas of bone resorption.[14]

In conclusion, combined CT and MRI provide accurate definition of chondrosarcoma extension and important diagnostic clues. However, biopsy is essential for histologic confirmation and treatment planning.

REFERENCES

1. Aretsky PJ, Kantu K, Freund HR, Polisar IA. Chondrosarcoma of the nasal septum. Ann Otol Rhinol Laryngol 1970; 79:382-8.

2. Gallagher TM, Strome M. Chondrosarcoma of the facial region. Laryngoscope 1972;82:978-84.

3. Gray R, Leonard G. Chondrosarcoma of the nasal septum. J Laryngol Otol 1977;91:427-31.

4. McCoy JM, McConnel FM. Chondrosarcoma of the nasal septum. Arch Otolaryngol 1981;107:125-7.

5. Bailey CM. Chondrosarcoma of the nasal septum. J Laryngol Otol 1982;96:459-67.

6. El Ghazali AM. Chondrosarcoma of the paranasal sinuses and nasal septum. J Laryngol Otol 1983;97:543-7.

7. Hornibrook J, Robertson S. Chondrosarcoma arising in the nasal septum. J Laryngol Otol 1983;97:1163-8.

8. Nishizawa S, Fukaya T, Inouye K. Chondrosarcoma of the nasal septum: a report of an uncommon lesion. Laryngoscope 1984;94:550-3.

9. Randall CJ, Gray R. Nasal septum chondrosarcoma. J Laryngol Otol 1984;98:635-8.

10. Beneck D, Seidman I, Jacobs J. Chondrosarcoma of the nasal septum: a case report. Head Neck Surg 1984;7:162-7.

11. Nishimura Y, Amano Y, Ogasawara H. Chondrosarcoma of the nasal septum: surgical consideration on Le Fort I osteotomy. Eur Arch Otorhinolaryngol 1993;250:59-62.

12. Hyde GE, Yarington T, Chu FW. Head and neck manifestation of Maffucci's syndrome: chondrosarcoma of the nasal septum. Am J Otolaryngol 1995;16:272-5.

13. Saito K, Unni K, Wollan P, Lund B. Chondrosarcoma of the jaw and facial bones. Cancer 1995;76:1550-8.

14. Rassekh CH, Nuss DW, Kapadia SB, Curtin HD, Weissman JL, Janecka IP. Chondrosarcoma of the nasal septum: skull base imaging and clinicopathologic correlation. Otolaryngol Head Neck Surg 1996;115:27-37.

15. Barrett AW, Hopper C, Speight PM. Oral presentation of secondary chondrosarcoma arising in osteochondroma of the nasal septum. Int J Oral Maxillofac Surg 1996;25:119-21.

16. Ertefai P, Moghimi M. Chondrosarcoma of the nasal septum. Eur Arch Otorhinolaryngol 1997;254:259-60.

CLINICAL AND RESEARCH FELLOWSHIP IN OTOLOGY AND NEUROTOLOGY

MASSACHUSETTS EYE AND EAR INFIRMARY

Fellowship training is offered for individuals who aspire to a full-time academic career. Two- and three-year fellowships are available. Three-year fellowships provide additional opportunities for basic research training, while the two-year fellowship will provide opportunities for clinical research. Board eligibility or certification is required.

For more information, contact:

Michael J. McKenna, MD
Dept of Otolaryngology
Massachusetts Eye and Ear Infirmary
243 Charles Street
Boston, MA 02114
(617) 573-3654

A2

Courses on the Lateral Approaches to the Skull Base

(In English)

Course Director:
Mario Sanna, MD

This cadaver hands-on workshop provides the individual opportunity to participate in:

- Video presentation of techniques and clinical cases
- Didactic presentations
- Performing the surgical procedures on specially prepared bones and half-heads
- Discussions

For additional information, contact:

Secretary Gruppo Otologico
Via Emmanueli n.42
29100 Piacenza - Italy
telephone: 0523/754362
fax: 0523/453708
E-mail: Corsi.Sanna@otologic.it

A161

IOWA BASIC SCIENCE COURSE IN OTOLARYNGOLOGY

A course for otolaryngology residents
Department of Otolaryngology–Head and Neck Surgery
The University of Iowa
Iowa City, Iowa
July 5 – August 24, 2001

Head and neck anatomy, under the instruction of Dr Robert Morecraft, includes daily anatomical lectures, supervised dissections, and individual assistance each session.

Lectures by specialists from the College of Medicine: Departments of Anesthesia, Dentistry, Dermatology, Internal Medicine, Neurology, Ophthalmology, Otolaryngology, Pathology, Pediatrics, Pharmacology, Radiology, and Surgery. The lecture series will review the following as they apply to otolaryngology: allergy, anatomy, anesthesia, audiology, biochemistry, craniofacial deformities, dentistry, epidemiology, evoked responses, genetics, immunology, instrumentation, nutrition, oncology, pharmacology, physics, physiology, psychoacoustics, radiology, research design, speech pathology, statistics, and trauma.

Fee: $2,850
Openings: Limited

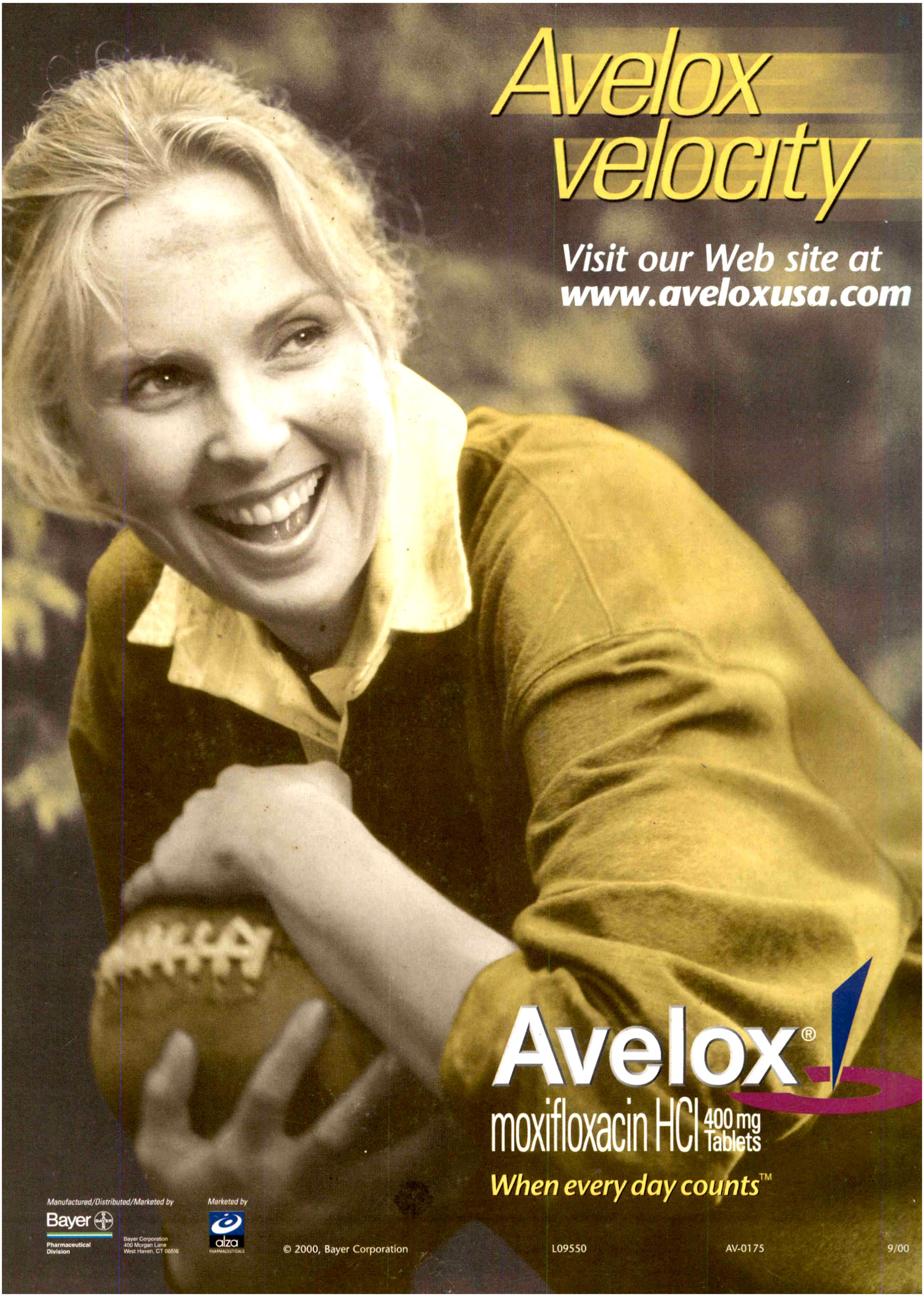
Coordinators: Margaret M. Browning, MD; Jay T. Rubinstein, MD; Richard S. Tyler, PhD

For further information, contact:

Margaret M. Browning, MD
Dept of Otolaryngology–Head and Neck Surgery
University of Iowa Hospitals and Clinics
200 Hawkins Drive, 21201 PFP
Iowa City, IA 52242-1093
Phone: (319) 356-4351; fax: (319) 356-4547; e-mail: margaret-browning@uiowa.edu
or visit our web site: http://www.medicine.uiowa.edu/otolaryngology/news/basiccourse.html

The University of Iowa does not discriminate in its educational programs and activities on the basis of race, national origin, color, religion, sex, age, or disability.

A142



© 2000, Bayer Corporation

VOLUME 110 (3) — MARCH 2001

—*Founded in 1892*—

ANNALS

OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

Official Organ of the
AMERICAN LARYNGOLOGICAL ASSOCIATION
AMERICAN BRONCHO-ESOPHAGOLOGICAL ASSOCIATIO

LECTIVE EXTRACORPOREAL MEMBRANE GENATION: AN IMPROVED PERIOPERATIVE HNIQUE IN THE TREATMENT OF TRACHEAL OBSTRUCTION

OLECULAR MARKERS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA: THEIR OLOGICAL FUNCTION AND PROGNOSTIC SIGNIFICANCE

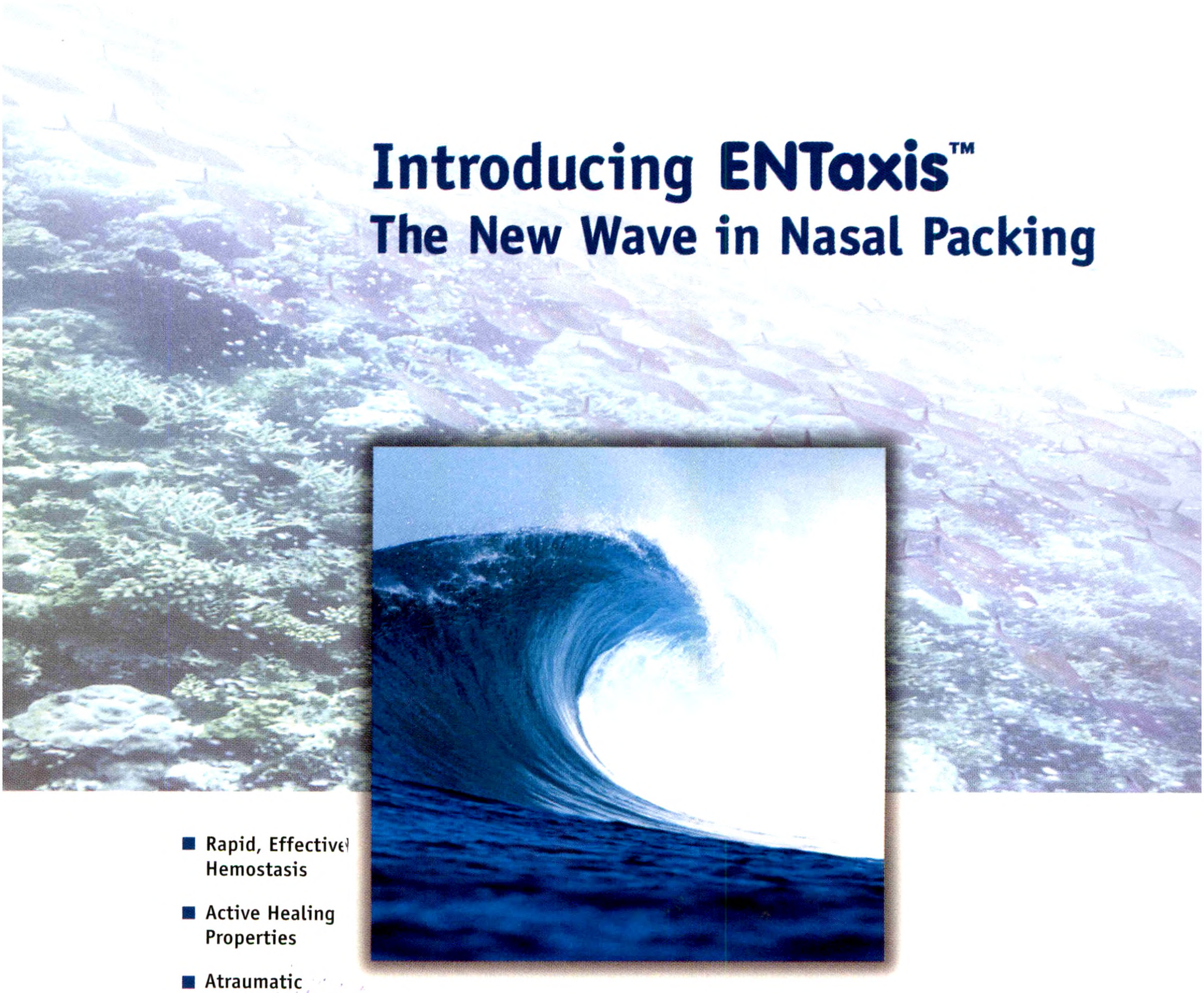
OBJECTIVE VOICE ANALYSIS AFTER OLOGOUS FAT INJECTION FOR UNILATERAL VOCAL FOLD PARALYSIS

UCOSA-ASSOCIATED LYMPHOID TISSUE IN MIDDLE EAR AND EUSTACHIAN TUBE

ENOID CYSTIC CARCINOMA OF THE BASE OF THE TONGUE

ANNALS
PUBLISHING CO.

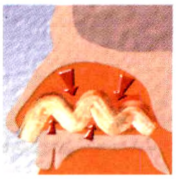
WEB SITE—http://www.Annals.com

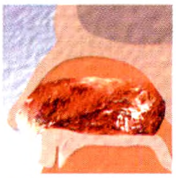


- Rapid, Effective Hemostasis
- Active Healing Properties
- Atraumatic Insertion and Removal

ENTaxis™ — The Only Nasal Packing with the Unique Chemical Properties of Seaweed

Solve the problems of managing epistaxis and post-surgical bleeding with ENTaxis™, the only nasal packing made from calcium alginate. A natural substance derived from seaweed, calcium alginate accelerates both hemostasis and healing.


ENTaxis™ is inserted easily...


...and expands 300%.

Soothing Science from the Sea

ENTaxis™ expands 300% for physical hemostasis, while also chemically activating platelet aggregation. In addition, ENTaxis™ actually gelates in the nasal cavity through ion exchange. So it maintains a moist environment and wicks secretions with continuous absorption. And the gelification makes removal virtually painless, with minimal risk of rebleeding.

Discover ENTaxis™ — the wave of the future in nasal packing. For a complimentary clinical sample, call 1-800-433-BMPI(2674) or visit us at www.bosmed.com.

Boston Medical Products

©2000 BMP Inc.

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

—Founded in 1892—

Editor

BRIAN F. McCABE, MD
Iowa City, Iowa

Associate Editors

CHARLES D. BLUESTONE, MD
Pittsburgh, Pa

GEORGE A. GATES, MD
Seattle, Wash

OLLIVIER LACCOURREYE, MD
Paris, France

Board of Editors

JOHN G. BATSAKIS, MD	Houston, Tex; Kalkaska, Mich
JOEL M. BERNSTEIN, MD, PhD	Buffalo, NY
ANDREW BLITZER, MD, DDS	New York, NY
DERALD E. BRACKMANN, MD	Los Angeles, Calif
POUL BRETLAU, MD, DMSc	Copenhagen, Denmark
ITZHAK BROOK, MD	Washington, DC
RICHARD A. BUCKINGHAM, MD	Park Ridge, Ill
RICHARD A. CHOLE, MD, PhD	St. Louis, Mo
ROBIN T. COTTON, MD	Cincinnati, Ohio
ALFIO FERLITO, MD	Udine, Italy
UGO FISCH, MD	Zurich, Switzerland
IMRE FRIEDMANN, MD, ScD	Middlesex, England
BRUCE GANTZ, MD	Iowa City, Iowa
LEE A. HARKER, MD	Omaha, Neb
JEFFREY P. HARRIS, MD, PhD	San Diego, Calif
GERALD B. HEALY, MD	Boston, Mass
LAUREN D. HOLINGER, MD	Chicago, Ill
WILLIAM F. HOUSE, MD	Los Angeles, Calif
STEVEN K. JUHN, MD	Minneapolis, Minn
TIMOTHY T. K. JUNG, MD, PhD	Loma Linda, Calif
DAVID W. KENNEDY, MD	Philadelphia, Pa
FRANK E. LUCENTE, MD	New York, NY
WILLIAM L. MEYERHOFF, MD, PhD	Dallas, Tex
JOSEF MILLER, PhD	Ann Arbor, Mich
AAGE R. MØLLER, PhD	Dallas, Tex
WILLIAM W. MONTGOMERY, MD	Boston, Mass
EUGENE N. MYERS, MD	Pittsburgh, Pa
JOSEPH B. NADOL, Jr, MD	Boston, Mass
YASUYA NOMURA, MD	Tokyo, Japan
LORNE S. PARNES, MD	London, Canada
RANDALL L. PLANT, MD	Norfolk, Va
JAI H. RYU, PhD	Winston-Salem, NC
ISAMU SANDO, MD	Pittsburgh, Pa
CLARENCE T. SASAKI, MD	New Haven, Conn
ROBERT T. SATALOFF, MD, DMA	Philadelphia, Pa
RICHARD J. H. SMITH, MD	Iowa City, Iowa
HEINZ R. STAMMBERGER, MD	Graz, Austria
DEAN M. TORIUMI, MD	Chicago, Ill
JAN E. VELDMAN, MD, PhD	Utrecht, The Netherlands
BRUCE M. WENIG, MD	Bronx, NY
T. J. YOO, MD, PhD	Memphis, Tenn
STEVEN M. ZEITELS, MD	Boston, Mass

Software Review and Computer Applications Editor

KEVIN T. KAVANAGH, MS, MD .... Somerset, Ky

Imaging Editor and Book Review Editor

DENNIS G. PAPPAS, MD .... Birmingham, Ala

Emeritus Board Members

WESLEY H. BRADLEY, MD	Albany, NY
JACK D. CLEMIS, MD	Chicago, Ill
SEYMOUR COHEN, MD	Los Angeles, Calif
CHARLES W. CUMMINGS, MD	Baltimore, Md
PATRICK J. DOYLE, MD	Vancouver, Canada
ARNDT J. DUVALL III, MD	Minneapolis, Minn
JOHN M. FREDRICKSON, MD	St Louis, Mo
RICHARD R. GACEK, MD	Syracuse, NY
JEROME C. GOLDSTEIN, MD	Lake Worth, Fla
SIR DONALD F. N. HARRISON, MD PhD	London, England
SAM E. KINNEY, MD	Cleveland, Ohio
JOHN A. KIRCHNER, MD	New Haven, Conn
DAVID J. LIM, MD	Los Angeles, Calif
FRED H. LINTHICUM, Jr, MD	Los Angeles, Calif
MICHEL PORTMANN, MD	Bordeaux, France
LORING W. PRATT, MD	Waterville, Me
FRANK N. RITTER, MD	Ann Arbor, Mich
ROBERT J. RUBEN, MD	Bronx, NY
JACOB SADÉ, MD	Tel Aviv, Israel
WILLIAM H. SAUNDERS, MD	Columbus, Ohio
RUEDIGER THALMANN, MD	St Louis, Mo
HARVEY M. TUCKER, MD	Cleveland, Ohio
PAUL H. WARD, MD	Los Angeles, Calif

Calcutta University College of Medicine C.U.
LIBRARY

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

— Founded in 1892 —

ANNALS
PUBLISHING CO.

EDITOR'S OFFICE

Brian F. McCabe, MD
Department of Otolaryngology –
Head & Neck Surgery
University Hospitals & Clinics
Iowa City, IA 52242
(319) 356-2310

BUSINESS OFFICE

Annals Publishing Company
4507 Laclede Avenue
St Louis, MO 63108
(314) 367-4987
Fax (314) 367-4988
E-mail: manager@Annals.com

STAFF

Managing Editor
Michol L. Sheridan

Business Manager
Kenneth A. Cooper, Jr

Chief Copyeditor
Edna Hayes Harrison

Copyeditor
Scott A. Bailey

Circulation Manager
Kevin R. Cooper

Editorial Assistant
Sarah G. Keith

ADVERTISING REPRESENTATIVES

— East & Midwest —
Cunningham Associates
180 Old Tappan Rd
Old Tappan, NJ 07675
(201) 767-4170
Fax (201) 767-8065
JPattis@cunnasso.com

— West —
Charles B. Healy
PO Box 5213
Palm Springs, CA 92263
(760) 320-0118

EDITORIAL CONTENT

The *ANNALS* publishes original manuscripts of clinical and research importance in otolaryngology – head and neck medicine and surgery, bronchoesophagology, laryngology, head and neck oncology and surgery, plastic and reconstructive surgery, pediatric otolaryngology, audiology, speech pathology, and related specialties. In-depth studies (supplements), papers of historical interest, reviews of computer software and applications in otolaryngology are also published, as well as imaging, pathology, and clinicopathology studies, book reviews, and letters to the editor. All papers and supplements are peer-reviewed. The *ANNALS* is the official journal of the American Laryngological Association and the American Broncho-Esophagological Association.

SUBSCRIPTIONS (INCLUDES ON-LINE SUBSCRIPTION)

2001 Annual Subscription Rates	*USA*	*International Surface Mail (All Regions)*
Institution*	$240.00	$265.00
Individual†	170.00	195.00
Resident/Student†	112.00	129.00

*Institutional (multiple-reader) subscription rates apply to public and private libraries, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and commercial and private institutions and organizations.

†Individual, resident, and student subscriptions must be in the individual's name and must be billed to and paid for by the individual.

Airmail rates are available upon request.

Visa, Mastercard, and American Express are accepted as payment.

WEB SITE

http://www.Annals.com

AVAILABILITY OF CD-ROM

CD-ROMs are available for each volume in March of the following year. The 1998 volume was the first on CD-ROM. Current subscribers can purchase a CD-ROM at $76.00 for an individual and $99.00 for an institution. Orders placed by non-subscribers are $175.00 for an individual and $245.00 for an institution.

CLAIMS & BACK ISSUES

Claims. Claims for missing issues must be received within 60 days of the month of publication for the United States and Canada, and 180 days for other countries. Single copy prices will be charged on missing issues claimed after that time.

Back Issues. A comprehensive inventory of back issues and supplements is available. Prices are furnished on request.

Change of Address. Address changes must be received 45 days before the effective date so that the subscription will be received without interruption.

MICROFILM

The *ANNALS* is available in microform from University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106.

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY (ISSN 0003-4894) is published monthly by Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108. Periodicals postage paid at St Louis, Mo. POSTMASTER: Send address changes to *ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY*, c/o Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108-2103.

COPYRIGHT© 2001, ANNALS PUBLISHING COMPANY

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

VOLUME 110 MARCH 2001 NUMBER 3

CONTENTS

FROM THE AMERICAN BRONCHO-ESOPHAGOLOGICAL ASSOCIATION

Elective Extracorporeal Membrane Oxygenation: An Improved Perioperative Technique in the Treatment of Tracheal Obstruction
Kieran M. Connolly, MD; William F. McGuirt, Jr, MD 205

Arytenoid Prolapse as a Consequence of Cricotracheal Resection in Children
Michael J. Rutter, FRACS; Dana Thompson Link, MD; Benjamin E. J. Hartley, FRCS; Robin T. Cotton, MD 210

Long-Term Monitoring of Respiration With a Mediastinal Pressure Sensor in Dogs
George S. Goding, Jr, MD; David W. Eisele, MD; Mark A. Christopherson, MS; Alan R. Schwartz, MD; Roy L. Testerman, PhD 215

INDEPENDENT CONTRIBUTIONS

Molecular Markers in Head and Neck Squamous Cell Carcinoma: Their Biological Function and Prognostic Significance
Benjamin D. Smith; Bruce G. Haffty, MD; Clarence T. Sasaki, MD 221

Objective Voice Analysis After Autologous Fat Injection for Unilateral Vocal Fold Paralysis
Dana M. Hartl, MD; Stéphane Hans, MD; Jacqueline Vaissière, PhD; Marc Riquet, MD; Ollivier Laccourreye, MD; Daniel F. Brasnu, MD 229

Enhancement of Submicroscopic Damage of the Nasal Epithelium by Topical Allergen Challenge in Patients With Perennial Nasal Allergy
Hiroshi Nagata, MD, PhD; Hideaki Motosugi, MD, PhD; Akiko Sanai, MD, PhD; Homare Suzuki, MD, PhD; Kazuto Ohno, MD, PhD; Tsutomu Numata, MD, PhD; Akiyoshi Konno, MD, PhD 236

Mucosa-Associated Lymphoid Tissue in Middle Ear and Eustachian Tube
Masami Kamimura, MD, DMSc; Isamu Sando, MD, DMSc; Carey D. Balaban, PhD; Shin-ichi Haginomori, MD, DMSc 243

Adenoid Cystic Carcinoma of the Base of the Tongue
Ali Namazie, MD; Sassan Alavi, MD; Elliot Abemayor, MD, PhD; Thomas C. Calcaterra, MD; Keith E. Blackwell, MD 248

Is Pressure Dressing Necessary After Ear Surgery?
Marco L. Castelli, MD, FRCS; Diego Di Lisi, MD; Paolo Marcato, MD; Paolo Tavormina, MD; Elena Cappellaro, MD; Alberto Sartoris, MD, PhD 254

Hypertrophic Neuropathy of the Facial Nerve
Romain E. Kania, MD; Dominique Cazals-Hatem, MD; Didier Bouccara, MD; Françoise Cyna-Gorse, MD; Fabrice Lisovoski, MD, PhD; Dominique Hénin, MD; Olivier Sterkers, MD, PhD 257

Bilateral Laryngeal Pseudoparalysis in Xanthoma Disseminatum Treated by Endoscopic Laser Medial Arytenoidectomy
Giovanna Cantarella, MD; Cesare Bartolomeo Neglia, MD; Angelo Valerio Marzano, MD; Antonio Ottaviani, MD 263

Tracheal Length of Infants Under Three Months Old
Kuo-Sheng Lee, MD; Cheng-Chien Yang, MD 268

Long-Term Changes in Off-Lesion Endocochlear Potential After Induction of Localized Lesions in the Lateral Wall
Rong Wu, MD, PhD; Tomoyuki Hoshino, MD, PhD 271

Ligand-Gated Purinergic Receptors Are Differentially Expressed in the Adult Rat Vestibular Periphery
James L. Kreindler, MD; Marta Troyanovskaya, PhD; Phillip A. Wackym, MD 277

Effect of Furosemide on Basal Lamina Anionic Sites in Guinea Pig Labyrinth
Mitsuya Suzuki, MD; Kimitaka Kaga, MD 283

IMAGING CASE STUDY OF THE MONTH

Rhinoscleroma Mimicking Nasal Polyposis
Mahmoud El-Sherif M. Ammar, MD; Arie Rosen, MD 290

MISCELLANEOUS

Information for Subscribers 2a
Index to Advertisers 8a

Ann Otol Rhinol Laryngol 110:2001

ELECTIVE EXTRACORPOREAL MEMBRANE OXYGENATION: AN IMPROVED PERIOPERATIVE TECHNIQUE IN THE TREATMENT OF TRACHEAL OBSTRUCTION

KIERAN M. CONNOLLY, MD WILLIAM F. MCGUIRT, JR, MD
WINSTON-SALEM, NORTH CAROLINA

The surgical management of children with tracheal stenosis and obstruction is complicated by the perioperative needs of pressure ventilation and indwelling endotracheal tubes. These factors predispose to surgical failure and anastomotic breakdown, restenosis, and pneumomediastinum. The use of extracorporeal membrane oxygenation (ECMO) to manage ventilation during tracheal repair allows better visualization at the surgical site and obviates the need for indwelling endotracheal tubes and high-pressure ventilation. Six children were treated with elective ECMO at a tertiary care hospital. All 6 underwent successful surgical repair, and 4 of the 6 were ultimately extubated. There were no significant complications at the surgical site. There was 1 death from postoperative complications, and 2 patients required tracheotomy. One tracheotomy was performed for upper airway obstruction secondary to retrognathia, and this patient was subsequently decannulated. Medical complications were confined to 2 patients and included sepsis, hyperbilirubinemia, seizure disorder, renal failure, intracranial hemorrhage, and hydrocephalus. Elective ECMO provides a reliable perioperative technique for airway management of children with tracheal stenosis or obstruction. This technique offers the advantage of improved visibility at the operative site and eliminates the need for high-pressure ventilation, thereby likely reducing the risk of perioperative morbidity.

KEY WORDS — extracorporeal membrane oxygenation, tracheal stenosis.

INTRODUCTION

The management of children with tracheal obstruction is complex and involves a considerable risk of morbidity and mortality. The surgical complications include anastomotic breakdown, pneumomediastinum, and restenosis.[1-4] Surgical failure can result from the presence of indwelling endotracheal tubes during the operation, as well as the use of perioperative high-pressure ventilation. In an attempt to avoid these complications, extracorporeal membrane oxygenation (ECMO) was used in 6 patients in the perioperative period. This technique involves the delivery of blood to a semipermeable membrane in which gas exchange takes place. It obviates the need for both intraoperative and postoperative high-pressure ventilation. The working premise was that the use of ECMO would improve the outcome and surgical success by allowing for better visualization of the operative site and by decreasing the stress that high-pressure ventilation places upon an anastomosis.

CASE REPORTS

Six children underwent repair of obstructing tracheal lesions with the use of elective ECMO in the perioperative period. Four of the patients had long-segment congenital tracheal stenosis. One patient had an obstructing duplication cyst of the middle part of the trachea, and 1 patient had an acquired, posttraumatic distal tracheal stenosis. The 6 cases are described in detail below.

Case 1. An infant was born at 34 weeks of gestational age to a mother who abused multiple substances. The patient had respiratory distress at birth, requiring endotracheal intubation. On day 6 of life, the patient underwent direct laryngoscopy and bronchoscopy, which revealed a tracheal stenosis secondary to complete tracheal rings. The stenotic area extended from the first tracheal ring to the distal third of the trachea. At 1 month of age, the patient underwent sternotomy with a sliding tracheoplasty. The patient was placed on ECMO during the procedure and remained on ECMO for the first 5 postoperative days. On postoperative day 7, the patient underwent direct laryngoscopy and bronchoscopy, which revealed the tracheal repair to be intact and without residual stenosis or granulation tissue present. After 3 unsuccessful attempts at extubation, the patient underwent tracheotomy. It was believed that the failure of extubation was the result of upper airway obstruction secondary to retrognathia. The patient subsequently underwent mandibular advancement and was decannulated.

From the Department of Otolaryngology–Head and Neck Surgery, Wake Forest University Baptist Medical Center, and Brenner Children's Hospital, Winston-Salem, North Carolina.

Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

CORRESPONDENCE — William F. McGuirt, Jr, MD, Dept of Otolaryngology–Head and Neck Surgery, Wake Forest University Baptist Medical Center, Medical Center Blvd, Winston-Salem, NC 27157.

Case 2. A 2-month-old infant presented with progressive stridor to a community hospital. The patient developed respiratory distress requiring intubation and was transferred to our tertiary care facility. Direct laryngoscopy and bronchoscopy revealed an obstructing duplication cyst of the middle part of the trachea. The cyst was confirmed by use of computed tomography and magnetic resonance imaging of the neck and chest. Two weeks after admission, the patient underwent sternotomy with partial segmental resection of the trachea with pericardial patch closure. The patient was placed on ECMO for the procedure and remained on ECMO for the first 72 hours after the operation. At 72 hours, the patient was converted to mechanical ventilation. After a period of 1 week, the patient underwent direct laryngoscopy and bronchoscopy, which revealed no evidence of granulation tissue or stenosis. The patient was extubated at that time. The patient remained extubated and was discharged home after an uneventful postoperative course.

Case 3. A term infant developed respiratory distress on day 2 of life. The pregnancy had been complicated by polyhydramnios. Bronchoscopy revealed tracheal stenosis with complete tracheal rings from the second ring to the level of the carina. An echocardiogram revealed a patent ductus arteriosus and a ventricular septal defect. The patient also had bronchopulmonary dysplasia. At 6 weeks of life, the patient underwent sternotomy with sliding tracheoplasty. The patent ductus arteriosus was repaired at that time. The patient was placed on ECMO during the procedure and remained on ECMO for the first 5 postoperative days. Bronchoscopy was performed 2 weeks after the operation and revealed mild to moderate tracheomalacia and an intact repair without granulation. The patient failed extubation attempts during postoperative weeks 2 and 4, but was successfully extubated during postoperative week 6. Her failure to be extubated was believed to be secondary to her intrinsic lung disease.

Case 4. A 37-weeks–gestational age infant was born with respiratory distress requiring intubation at birth. The pregnancy had been complicated by cocaine abuse, lack of prenatal care, and maternal hypertension and gestational diabetes. On day 2 of life, the patient underwent a second unsuccessful extubation and continued to have respiratory distress after reintubation. The patient was taken emergently to the operating room and underwent direct laryngoscopy and bronchoscopy, which revealed an obstructing mucous crust and tracheal stenosis secondary to complete tracheal rings. The stenotic segment extended from the fourth tracheal ring to 0.5 cm above the carina. At week 3 of life, the patient underwent sternotomy with a sliding tracheoplasty and a pericardial patch graft. The patient was on ECMO during the procedure and for 8 days afterward. The patient had a prolonged postoperative course that was complicated by intracranial hemorrhage, hydrocephalus, sepsis, and hyperbilirubinemia. The patient underwent bronchoscopy 2 weeks after the operation, which revealed a widely patent trachea. The patient was also noted to have a left vocal cord paralysis, which resolved during the patient's hospital stay. The patient underwent multiple unsuccessful attempts at extubation and remained mechanically ventilated for a total of 121 days. Repeat bronchoscopy 3 months after the operation revealed mild upper tracheal narrowing. The patient's intrinsic lung disease prevented weaning from the ventilator, and ultimately, the patient required a tracheotomy. One month after the tracheotomy, the patient was discharged.

Case 5. A 31-weeks–gestational age premature infant developed respiratory distress shortly after birth, requiring intubation. The pregnancy had been complicated by quadruple gestation and gestational diabetes. At week 3 of life, the patient was extubated, but required reintubation 4 days later. On week 4 of life, the patient underwent direct laryngoscopy and bronchoscopy, which revealed a tracheal stenosis that extended from the middle of the trachea to the level of the carina. The patient had multiple comorbidities, including a patent foramen ovale, a double outlet right ventricle, and a ventricular septal defect. Before the tracheal repair, the patient had developed anuria, which resolved after a clot was passed. Ultrasonography performed at that time revealed a single kidney. The patient had also had 2 episodes of respiratory arrest secondary to endotracheal tube obstruction, as well as poor growth. The multidisciplinary team involved in the patient's care decided at this time that the only chance of survival was to proceed with tracheal repair. At 2 months of life, the patient underwent a sliding tracheoplasty with a pericardial patch graft. The patient was placed on ECMO for the surgical repair and remained on ECMO for 9 postoperative days. Bronchoscopy performed on postoperative day 9 revealed the airway to be widely patent, with no evidence of anastomotic breakdown. There was minimal granulation tissue present at the repair site. The patient's postoperative course was complicated by sepsis, renal failure, hyperbilirubinemia, and seizure disorder. The patient died during postoperative week 3.

Case 6. A 10-year-old, healthy boy was involved in a bicycle accident in which he was struck by a motor vehicle. The patient had been evaluated and treated at an outside hospital. He was found to have a subdural hematoma and required mechanical ven-

PATIENT DATA

Case	Diagnosis	*ECMO Use*	*Surgical Repair*	*Comorbidities*	*Complications*	
					At Anastomosis	*Other*
1	Long-segment tracheal stenosis, complete tracheal rings	Intraoperative, 5 days postoperative	Sliding tracheoplasty	Prematurity (34-week gestation)	None	Required tracheotomy
2	Duplication cyst of middle part of trachea	Intraoperative, 72 hours postoperative	Segmental resection with pericardial patch graft	None	None	None
3	Long-segment tracheal stenosis, complete tracheal rings	Intraoperative, 5 days postoperative	Sliding tracheoplasty	Bronchopulmonary dysplasia, ventriculoseptal defect, patent ductus arteriosus	Mild tracheomalacia	Required tracheotomy
4	Long-segment tracheal stenosis, complete tracheal rings	Intraoperative, 7 days postoperative	Sliding tracheoplasty with pericardial patch graft	Maternal gestational cocaine abuse, gestational diabetes, gestational hypertension	Mild upper tracheal narrowing	Left vocal cord paralysis (temporary), intracranial hemorrhage, hydrocephalus, sepsis, hyperbilirubemia
5	Long-segment tracheal stenosis	Intraoperative, 9 days postoperative	Sliding tracheoplasty with pericardial patch graft	Prematurity (31-week gestation), quadruple gestation, gestational diabetes, patent foramen ovale, double outlet right ventricle, ventriculoseptal defect, unilateral kidney with temporary preoperative renal insufficiency	Minimal granulation	Sepsis, renal failure, hyperbilirubinemia, seizure disorder
6	Short-segment tracheal stenosis	Intraoperative	Segmental resection with end-to-end anastomosis	None	None	None

tilation for a period of 48 hours. His mental status did not improve for a period of 2 weeks. After this 2-week period of convalescence, he was readmitted for drainage of his subdural hematoma. After the drainage, he developed stridor, which progressively worsened after discharge. He was admitted to this facility, and a midtracheal stenosis was found on a computed tomography scan. He was placed on heliox (a combination of helium and oxygen) to avoid intubation prior to definite repair. The patient subsequently underwent bronchoscopy, which confirmed a stenosis involving a short segment 4 cm above the carina. He then underwent resection of the stenotic area and repair with an end-to-end anastomosis and a pericardial patch graft. During the operation, ECMO was used. The patient has remained asymptomatic since the time of the repair.

RESULTS

Six patients underwent repair of their obstructing tracheal lesions (see Table). Four of the 6 patients had long-segment tracheal stenosis secondary to complete tracheal rings. One patient had a short-segment stenosis, and 1 patient had an obstructing duplication cyst. During the repair, ECMO was used in all 6 cases, and it was used after the repair in 5 of the 6 cases. The duration of postoperative ECMO therapy varied from 72 hours to 9 days. There were no significant complications at the surgical site. There was 1 case of mild tracheomalacia, 1 case of mild upper airway narrowing, and 1 case in which a small amount of granulation tissue was found at the anastomotic site. Ultimately, 4 of the 6 patients were successfully extubated and discharged. Two patients required tracheotomy. One of the 2 patients who underwent tracheotomy was successfully decannulated after treatment of upper airway obstruction resulting from retrognathia. There were multiple postoperative medical complications, but they were confined to 2 patients. One of these patients subsequently died. The complications included sepsis, hyperbilirubinemia, seizure disorder, renal failure, intracranial hemorrhage, and hydrocephalus.

DISCUSSION

The treatment of patients with obstructing tracheal lesions, particularly long-segment lesions, can be fraught with complications. Until 1982, long-segment congenital tracheal stenosis was regarded by some as a fatal diagnosis.[5,6] Improvements in surgical techniques, including sliding tracheoplasties, omental pedicle flaps, pericardial patch grafts, and cartilage grafts, have made treatment possible.[1,2,5,6] The rates of morbidity and mortality associated with reconstruction remain high. One of the largest series was reported by Loeff et al[2] in 1988. In this series of 22 patients, the overall mortality rate was 77%. The complications in their series included tracheal air leaks, bronchopulmonary fistula, and pulmonary hemorrhage.[1-4]

The difficulties associated with surgical repair are multiple. Among the greatest difficulties are those associated with intraoperative and postoperative ventilation. Traditionally, ventilation in the intraoperative period was carried out either through an endotracheal tube passed through the obstruction or by cannulation of the airway distal to the obstructing lesion with subsequent removal.[1,6,7] These techniques can obscure the operative site and, in patients with lesions of the trachea that may narrow it to less than 3 mm, can create a significant challenge to surgical repair. In the postoperative period, the endotracheal tube must remain in place — potentially, for weeks — and even small air leaks around the endotracheal tube can place considerable stress upon the anastomotic site. This problem is of particular concern in patients with underlying lung disease, who may require significant pressures for adequate ventilation. Additionally, an accidental extubation would in all likelihood lead to emergent reintubation with potential disruption of the surgical site. Treatment of distal tracheal lesions can be even more dangerous, in that cannulation of the distal airway may be difficult or impossible. This is particularly true of lesions that extend down to the carina.

In an attempt to avoid the need for mechanical ventilation, cardiopulmonary bypass and, more recently, the use of ECMO have been reported.[1-3,7] These techniques to date have focused mainly on intraoperative management. Cardiopulmonary bypass is significantly more invasive than ECMO, in that it generally requires cannulation of the aorta as well as the right atrium. In addition, the relatively large catheters are typically within the operative field and may obscure the view of the surgical site.[7] Clowes et al[8] first described the use of ECMO in 1956, although the first clinical trials of prolonged ECMO were not completed until the 1970s.[9,10] The survival rates were initially poor and have improved only modestly. The studies involved critically ill patients who were presumed to be terminally ill without the use of ECMO; thus, even modest survival rates were deemed successes.[9,11] The reported uses of ECMO in neonates have included treatment of persistent pulmonary hypertension, meconium aspiration, diaphragmatic hernia, sepsis, and respiratory distress syndrome.[9,11] Extracorporeal membrane oxygenation may be either arteriovenous (cannulation of a major artery and vein) or venovenous (cannulation of 2 major veins). Blood is delivered through a semipermeable membrane in which oxygen transfer takes place.

The use of ECMO to facilitate repair of a distal tracheal stenosis was reported by Walker et al[7] in 1992. The case involved a patient with a 1.5-cm distal tracheal stenosis. The patient's postoperative course was complicated by pleural effusions and the need for tracheotomy for tracheomalacia. As demonstrated in this case, the technique is particularly applicable to distal tracheal lesions; it may, however, also facilitate repair and postoperative management of any tracheal lesion requiring an open technique. In this series, 6 patients underwent surgical repair of their tracheal lesions using ECMO. In 5 of the patients, ECMO was used in both intraoperative and postoperative management. The results at the surgical site were promising, in that all 6 patients had a widely patent airway, and only 1 patient required subsequent endoscopic excision of granulation tissue at the operative site. Two of the patients required tracheotomy. One of these patients was successfully decannulated after mandibular advancement surgery to alleviate upper airway obstruction. One patient had a transient left vocal cord paralysis.

The use of ECMO should not be regarded as a panacea with regard to open airway procedures in neonates and children. There may be significant morbidity associated with the use of ECMO. Potential complications include bleeding, sepsis, embolism, renal failure, and seizures, as well as other neurologic injury.[7,11] In this series, 2 patients had significant postoperative complications. One of the 2 patients ultimately died. It is unclear what role the use of ECMO may have played in the development of these complications. Both patients had multiple comorbidities, and 1 was critically ill before the operation.

CONCLUSION

The use of elective ECMO both during operation and in the immediate postoperative period may contribute to improved outcomes in the surgical treatment of obstructing tracheal lesions. In this series, 6 patients underwent surgical repair of obstructing tracheal lesions. The surgical outcomes were promising, in that only 1 of the 6 patients demonstrated granulation tissue at the anastomotic site after the operation. Four of the 6 patients had successful long-term outcomes, with 1 death and 1 patient requiring long-term tracheotomy. The use of elective ECMO should be considered in obstructing tracheal lesions requiring open repair. It may decrease the technical difficulty of the surgery, as well as the morbidity and mortality. Further studies regarding specific indications and outcomes are warranted.

REFERENCES

1. Tsugawa C, Nishijima E, Muraji T, et al. The use of omental flap for tracheobronchial reconstruction in infants and children. J Pediatr Surg 1991;26:762-5.

2. Loeff DS, Filler RM, Ein VS, Williams WG, Smith CR, Bahoric A. Congenital tracheal stenosis: a review of 22 patients from 1965 to 1987. J Pediatr Surg 1988;23:744-8.

3. Ein SH, Friedburg J, Williams WG, Rearon B, Barker GA, Mancer K. Tracheoplasty. A new operation for complete congenital tracheal stenosis. J Pediatr Surg 1982;17:872-8.

4. Grillo HC, Zannini P, Michelassi F. Complications of tracheal reconstruction: incidence, treatment, and prevention. J Thorac Cardiovasc Surg 1986;91:322-8.

5. Tsugawa C, Kimura K, Muraji T, Nishijima E, Matsumoto Y, Murata H. Congenital stenosis involving a long segment of the trachea: further experience in reconstructive surgery. J Pediatr Surg 1988;23:471-5.

6. Kimura K, Mukohara N, Tsugawa C, et al. Tracheoplasty for congenital tracheal stenosis of the entire trachea. J Pediatr Surg 1982;17:869-71.

7. Walker LK, Wetzel RC, Haller JA. Extracorporeal membrane oxygenation for perioperative support during congenital tracheal stenosis repair. Anesth Analg 1992;75:825-9.

8. Clowes GH, Hopkins AL, Neville WE. An artificial lung dependent upon diffusion of oxygen and carbon dioxide through plastic membranes. J Thorac Surg 1956;32:630-7.

9. Short BL, Pearson GD. Neonatal extracorporeal membrane oxygenation: a review. J Intensive Care Med 1986;1:47-54.

10. Hill DJ, O'Brien GO, Murray JJ. Prolonged extracorporeal oxygenation for acute post-traumatic respiratory failure. N Engl J Med 1972;286:629-34.

11. Stolar CJ, Snedecor SM, Bartlett RH. Extracorporeal membrane oxygenation and neonatal respiratory failure: experience from the Extracorporeal Life Support Organization. J Pediatr Surg 1991;26:563-71.

Calcutta University College of Medicine C. U.
LIBRARY

Ann Otol Rhinol Laryngol 110:2001

ARYTENOID PROLAPSE AS A CONSEQUENCE OF CRICOTRACHEAL RESECTION IN CHILDREN

MICHAEL J. RUTTER, FRACS
CINCINNATI, OHIO
BENJAMIN E. J. HARTLEY, FRCS
LONDON, ENGLAND

DANA THOMPSON LINK, MD
ROCHESTER, MINNESOTA
ROBIN T. COTTON, MD
CINCINNATI, OHIO

Cricotracheal resection (CTR) is a technique introduced comparatively recently for treating severe laryngotracheal stenosis in children. The recognized complications of CTR include recurrent laryngeal nerve damage, anastomotic dehiscence, and restenosis. We describe a further complication of CTR, namely, prolapse of the arytenoid cartilage. The presentation may be late, with symptoms of shortness of breath on exertion and nocturnal stertor with a poor sleep pattern, or the prolapse may be an asymptomatic incidental finding. The diagnosis is performed with flexible nasopharyngoscopy with the patient unanesthetized, or with rigid endoscopy with the patient lightly anesthetized and spontaneously ventilating. The affected arytenoid cartilage is noted to prolapse anteriorly and medially with inspiration, partly obstructing the airway. If treatment is required, endoscopic laser partial arytenoidectomy is effective. In a series of 44 children who underwent CTR, 20 were noted to develop arytenoid prolapse after operation. Twelve were asymptomatic, and 8 required laser arytenoidectomy, 2 of whom now require continuous positive airway pressure for moderate supraglottic collapse.

KEY WORDS — arytenoid prolapse, cricotracheal resection, pediatric airway.

INTRODUCTION

Cricotracheal resection (CTR) is a relatively new method for management of severe laryngotracheal stenosis in children. The technique involves resection of the anterior cricoid arch and thinning out of the posterior cricoid plate, with preservation of a mucosal flap.[1,2] The transected normal trachea is then telescoped into the posterior cricoid plate and anastomosed to the mucosal flap and the thyroid cartilage. This technique is well described in adults[3-6] and is increasingly recognized as a useful alternative to laryngotracheal reconstruction (LTR) utilizing expansion cartilage grafting in the management of severe laryngotracheal stenosis in children.[1,2,7-9] It is our experience that CTR has found particular application in the management of laryngotracheal stenosis when previous LTR has failed.

Cricotracheal resection is a more technically challenging procedure than standard expansion LTR, and there are recognized complications associated with the procedure. The previously described complications include damage to the recurrent laryngeal nerve, dehiscence of the anastomotic site, restenosis, and anastomotic web formation.[1,2,7] Arytenoid prolapse as a consequence of CTR has not been previously described. Recently, we described arytenoid prolapse as a consequence of LTR, and in our series we found a strong association with previous splitting or cartilage grafting of the posterior cricoid plate.[10] Children with arytenoid prolapse as a consequence of CTR were excluded from that review.

MATERIALS AND METHODS

Children who had undergone CTR at our institution and who had a follow-up period of at least 1 year were included in the analysis. A retrospective case review was performed, and the data gathered included a history of airway reconstruction prior to or subsequent to CTR. Any observations of arytenoid prolapse either prior to or following CTR were noted, as were presenting signs, symptoms, and interventions. Diagnosis required significant movement of the arytenoid cartilage both anteriorly and medially during inspiration (Fig 1). The arytenoid prolapse was noted during light general anesthesia with a spontaneous ventilation technique, and in virtually all cases, it was also noted on awake flexible nasopharyngoscopy.

RESULTS

During the period 1993 through 1998, CTR was performed in 44 children. All of these children had follow-up of at least 1 year; no child was lost to follow-up. Forty-two of the children had a grade III or

---

From the Department of Pediatric Otolaryngology, Children's Hospital Medical Center, Cincinnati, Ohio.

Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

CORRESPONDENCE — Michael J. Rutter, FRACS, Dept of Pediatric Otolaryngology, Children's Hospital Medical Center, Cincinnati, OH 45229-3039.

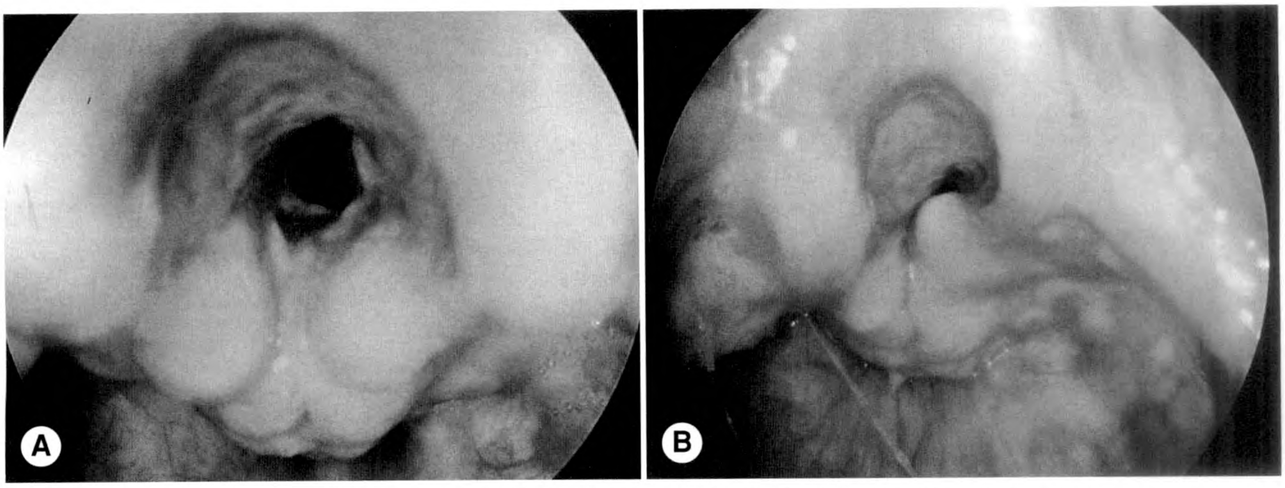


Fig 1. Endoscopic views of A) arytenoids during expiration and B) arytenoid prolapse during inspiration.

IV subglottic stenosis before the CTR. Thirty-five children had a history of LTR before CTR, and in 25 cases, there had been between 2 and 8 LTRs. In 15 cases, there was a history of a previous posterior cricoid split or posterior cricoid graft.

The demographics of the 44 children included an age range at operation of 13 months to 19 years, with a mean of 73 months. The male-to-female ratio was 25:19; 24 children had been born prematurely, and 14 children were on antireflux therapy at the time of CTR. A further 6 had previously undergone Nissen fundoplication. None of these factors influenced the likelihood of arytenoid prolapse (see Table).

Fourteen children had a history of arytenoid prolapse before CTR. All 14 had previously undergone LTR, with 10 having undergone at least 1 previous posterior cricoid split or posterior cricoid graft. Three of these children also had a history of laryngotracheoesophageal cleft, which in itself is a risk factor for arytenoid prolapse. Of the 14 children, 4 had a history of previous arytenoid surgery before CTR. Four had arytenoid surgery at the time of CTR for arytenoid prolapse (1 excision, 2 lateralizations, and 1 laser procedure). One further child had undergone right laser partial arytenoidectomy before CTR, and had a left laser partial arytenoidectomy at the time of CTR. In the remaining 5 children, the arytenoid prolapse was thought to be noncontributory to the airway obstruction, and no intervention was undertaken. This included 2 of the 3 children with a history of laryngeal clefting.

PATIENT DEMOGRAPHICS

		AP	*No AP*	*p* (χ^2 *test*)
Sex	Male	10	15	.597
	Female	10	9	
Prematurity	Yes	14	11	.192
	No	6	13	
GER	Yes	12	10	Approx 1
	No	6	11	
	Unknown	2	3	
SR	Yes	15	11	.05
	No	5	13	
PCS	Yes	10	9	.597
	No	10	15	
CPD	Yes	9	12	Approx 1
	No	10	11	
	Unknown	1	1	

AP — arytenoid prolapse, GER — history of gastroesophageal reflux, SR — suprahyoid release, PCS — posterior cricoid split, CPD — high posterior cricoid plate drill-out.

After CTR, arytenoid prolapse was noted in 25 children. Of these 25 patients, 14 had not had arytenoid prolapse noted before CTR, and in 6 children, the contralateral arytenoid cartilage was noted to prolapse after CTR. Therefore, in 20 children, arytenoid prolapse was noted after CTR, and it had not been present on that side before CTR.

The arytenoid prolapse often presented abruptly, and in 2 cases, the parents could name the exact day the symptoms appeared. The most common presenting symptoms were stridor (5), particularly with exertion, increased work of breathing during an attempted tracheotomy tube plugging trial (3), and disturbed sleep (3). One child presented with intermittent aphonia as the arytenoid cartilage prolapsed between the vocal cords, preventing full glottic closure. No other children presented with voice deterioration associated with the arytenoid prolapse. Arytenoid prolapse did not influence swallowing in any child.

The symptoms often were intermittent. Twelve of the 20 children in whom the arytenoid prolapse was noted only after CTR had no symptoms, or minimal symptoms that did not require intervention. All of the 8 children with symptoms required laser arytenoid

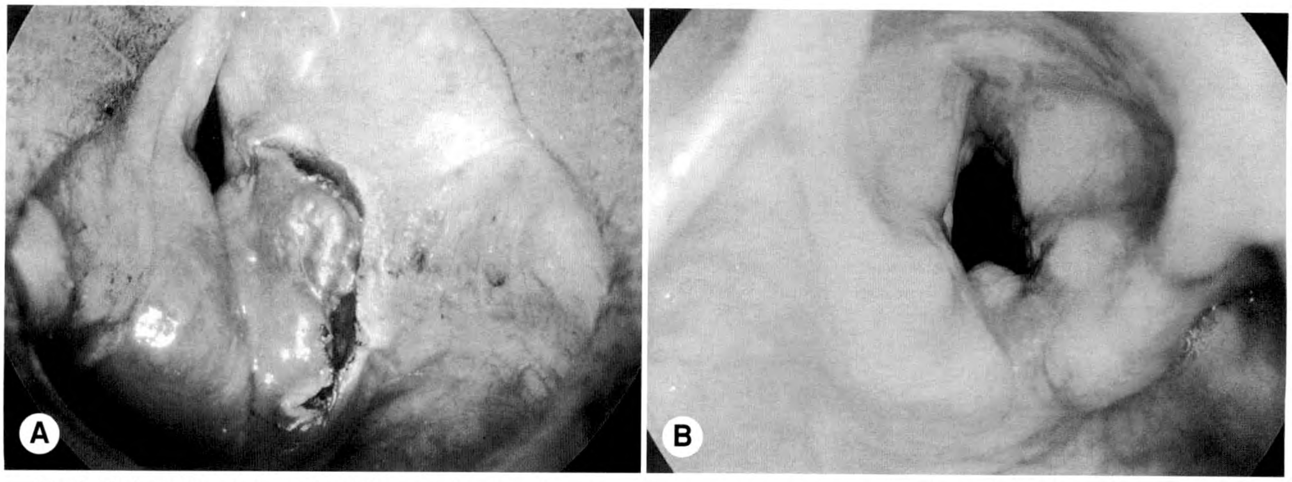


Fig 2. Endoscopic views of A) right partial arytenoidectomy with mucosal preservation utilizing laser flap technique and B) same patient's status 1 month after partial arytenoidectomy.

surgery (Fig 2). All were successfully decannulated, but 2 require nocturnal continuous positive airway pressure because of moderate supraglottic collapse. This was at least partly due to the laser surgery.

The time between CTR and the presentation of arytenoid prolapse varied between 2 and 50 months. The mean was 15 months. There was no significant difference between the group of patients who required surgical intervention and those who did not.

Other factors that may have contributed to arytenoid prolapse after CTR were examined. Neither a history of a past posterior cricoid split (or graft) nor the extent of the posterior cricoid drill-out during CTR was found to be an influence (see Table). A statistically significant association was seen in children who had a suprahyoid release: 26 children had a suprahyoid release, of whom 15 had subsequent arytenoid prolapse (58%), and 18 children did not have a suprahyoid release, of whom only 5 had subsequent arytenoid prolapse (28%; $p = .05$ on χ^2 test).

DISCUSSION

Arytenoid prolapse is not a well-described entity.[11-13] A recent review of our experience with arytenoid prolapse suggested that the most common etiologic factor was a history of LTR, particularly if there has been a previous posterior cricoid split or posterior cricoid cartilage graft.[10] However, we have also seen arytenoid prolapse associated with laryngotracheoesophageal clefts, upper airway burns, neurologic compromise, and tumors of the arytenoids (particularly lymphangiomas). We consider arytenoid prolapse associated with laryngomalacia to be a different entity.

An appreciation of the anatomy of the cricoarytenoid joint is essential in understanding the possible mechanisms that may cause instability at the joint. The facet of the cricoarytenoid joint faces obliquely upward, forward, and laterally on the cricoid arch. The only ligament stabilizing the arytenoid on the cricoid is the posterior cricoarytenoid ligament, which in fact lies medially (Fig 3). The posterior cricoarytenoid ligament is dynamically balanced by the action of the lateral cricoarytenoid muscle. This allows the joint to have 3 components of movement, namely, sliding, rocking, and twisting (on the fulcrum of the posterior cricoarytenoid ligament).[14-16]

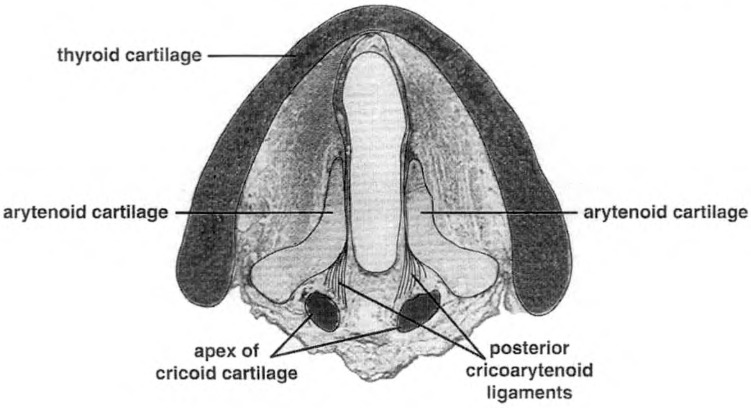


Fig 3. Position of posterior cricoarytenoid ligaments.

With LTR, a posterior cricoid split may promote instability of the joint and prolapse if 1) the posterior cricoid split is not exactly in the midline, as damage to the posterior cricoarytenoid ligament may occur, or 2) lateral distraction of the posterior plate of the cricoid distracts the two cricoarytenoid joints laterally, and therefore the bases of the arytenoid cartilages are also carried laterally. In the latter situation, the apices of the arytenoid cartilages, including the cuneiform and corniculate cartilages, maintain their more medial position, especially if the interarytenoid muscles are intact. This tendency will predispose the apices of the arytenoid cartilages to tilt medially, possibly, in turn, destabilizing the cricoarytenoid joints.

During CTR, extensive drilling of the posterior cricoid plate may either directly damage the cricoarytenoid joint, or damage the posterior cricoarytenoid ligament stabilizing the joint. An alternative explanation is that the resection of the anterior cricoid ring will usually sacrifice the lateral cricoarytenoid muscle, thereby removing the main counterbalance to the posterior cricoarytenoid ligament. Removal of the counterbalance will destabilize the joint, as the arytenoid cartilage is now prone to be displaced anteriorly and medially.

Suprahyoid release was associated with arytenoid prolapse; the anatomic reason for this association is not clear. The most likely explanation is that we perform suprahyoid releases less frequently now than we did at the beginning of the series. Because arytenoid prolapse may present late, and the children who have not had a suprahyoid release usually have had their CTR more recently, prolapse may simply not have presented yet in these children.

Arytenoid prolapse is a dynamic disorder; virtually all cases of prolapse occur during inspiration, because of the Bernoulli effect. Therefore, any limitation to airflow through the supraglottis and glottis may disguise the presentation. In our experience, the presence of a tracheotomy tube may disguise arytenoid prolapse, and unexpected obstructive symptoms during a plugging trial, in the presence of a statically adequate airway, may be the first clue to the diagnosis. The diagnosis is best made either with flexible nasopharyngoscopy or with rigid endoscopy using a spontaneous ventilation technique and light anesthesia. Both techniques depend upon good glottic airflow, so if a tracheotomy tube is present, it may need to be downsized and occluded to elicit the prolapse.

This difficulty in diagnosis is the reason that 2 children in this series with mild arytenoid prolapse noted before CTR did not initially seem to need intervention. The presence of the tracheotomy tube and a subglottic stenosis greatly limits airflow through the glottis and supraglottis, thus minimizing the Bernoulli effect and thereby the prolapse of the arytenoid. Subsequent reconstruction of the airway, with decannulation or tracheotomy tube plugging, greatly increases the airflow through the glottis and supraglottis, allowing more marked prolapse of the arytenoid cartilage, with symptomatic obstruction of the airway.

In this series of 44 patients, 20 presented with arytenoid prolapse following CTR that had not been noted before CTR. Although we have discussed the arytenoid prolapse in these cases as a consequence of CTR, the majority of these children had preexisting risk factors for arytenoid prolapse. These included previous LTR and a history of laryngotracheoesophageal cleft. Therefore, these children were predisposed to arytenoid prolapse before CTR, and the arytenoid prolapse may have been disguised by the presence of the tracheotomy and by the subglottic stenosis limiting airflow through the glottis. However, in 6 of the cases of arytenoid prolapse following CTR, there was no history of a laryngotracheoesophageal cleft or LTR. Also, the risk of arytenoid prolapse following CTR seems higher than the risk for arytenoid prolapse following LTR.

Although there is a high incidence of arytenoid prolapse following CTR, this group of children had severe subglottic stenosis, usually with a history of failed previous LTR. Therefore, given the high decannulation rate, we believe that the benefit of CTR outweighs the risk of subsequent arytenoid prolapse, particularly as a laser partial arytenoidectomy procedure is an extremely effective intervention for this problem. It is worth noting, however, that in 2 cases, after the laser treatment of the arytenoid or supraglottis, moderate supraglottic collapse required the use of nocturnal continuous positive airway pressure.

CONCLUSION

Arytenoid prolapse is a common sequela of CTR that has not previously been reported. It may be an asymptomatic finding, or it may present with symptoms of airway obstruction that require surgical intervention. The usual symptoms are exertional dyspnea or stridor and nocturnal restlessness, snoring, or apnea. The presentation may be late, and in some cases, the symptoms may be intermittent. Diagnosis is best accomplished with flexible nasopharyngoscopy in the office setting, or rigid endoscopy under light general anesthesia with a spontaneous ventilation technique. If intervention is required, laser partial arytenoidectomy is effective in the majority of cases. The procedure, however, carries a risk of supraglottic stenosis or collapse, and excision of tissue should be kept to a minimum.

REFERENCES

1. Monnier P, Savary M, Chapuis G. Partial cricoid resection with primary tracheal anastomosis for subglottic stenosis in infants and children. Laryngoscope 1993;103:1273-83.

2. Stern Y, Gerber ME, Walner DL, Cotton RT. Partial cricotracheal resection with primary anastomosis in the pediatric age group. Ann Otol Rhinol Laryngol 1997;106:891-6.

3. Ogura JH, Powers WE. Functional restitution of traumatic stenosis of the larynx and pharynx. Laryngoscope 1964;74:1081-110.

4. Gerwat J, Bryce DP. The management of subglottic laryngeal stenosis by resection and direct anastomosis. Laryngoscope 1974;84:940-57.

5. Pearson FG, Cooper JD, Nelems JM, Van Nostrand AWP. Primary tracheal anastomosis after resection of the cricoid cartilage with preservation of recurrent laryngeal nerves. J Thorac Cardiovasc Surg 1975;70:806-16.

6. Grillo HC, Mathieson DJ, Wain JC. Laryngotracheal resection and reconstruction for subglottic stenosis. Ann Thorac Surg 1982;53:54-63.

7. Monnier P, Lang F, Savary M. Partial cricotracheal resection for severe pediatric subglottic stenosis: update of the Lausanne experience. Ann Otol Rhinol Laryngol 1998;107:961-8.

8. Walner DL, Stern Y, Cotton RT. Margins of partial cricotracheal resection in children. Laryngoscope 1999;109:1607-10.

9. Vollrath M, Freihorst J, von der Hardt H. Surgery of acquired laryngotracheal stenoses in childhood. Experiences and results from 1988-1998. II: The cricotracheal resection [in German]. HNO 1999;47:611-22.

10. Rutter MJ, Liu JH, Link DT, Cotton RT. Arytenoid prolapse as a consequence of laryngotracheoplasty [Abstract]. Otolaryngol Head Neck Surg 1999;121:P150.

11. Reber A, Paganoni R, Frei FJ. Airway obstruction due to arytenoid prolapse in a child. Acta Anaesthesiol Scand 1999;43:104-6.

12. Turner GD, McGown RG. Airway obstruction due to arytenoid prolapse in two children [Letter]. Anaesthesia 1996;51:796.

13. Walner DL, Holinger LD. Supraglottic stenosis in infants and children. A preliminary report. Arch Otolaryngol Head Neck Surg 1997;123:337-41.

14. Sellars IE. A re-appraisal of intrinsic laryngeal muscle action. J Otolaryngol 1978;7:450-6.

15. Sellars IE. The anatomy and movements of the cricoarytenoid joint. Laryngoscope 1978;88:667-74.

16. McIlwain JC. The posterior glottis. J Otolaryngol 1991;20 (suppl 2).

LONG-TERM MONITORING OF RESPIRATION WITH A MEDIASTINAL PRESSURE SENSOR IN DOGS

GEORGE S. GODING, JR, MD
MINNEAPOLIS, MINNESOTA

DAVID W. EISELE, MD
BALTIMORE, MARYLAND

ALAN R. SCHWARTZ, MD
BALTIMORE, MARYLAND

MARK A. CHRISTOPHERSON, MS
MINNEAPOLIS, MINNESOTA

ROY L. TESTERMAN, PHD
MINNEAPOLIS, MINNESOTA

The ability of an implanted mediastinal pressure sensor to produce a stable respiratory signal that could be used to trigger stimulation of upper airway muscles was examined. In 6 dogs, a pressure sensor was secured to the manubrium (4 by wires and 2 by transmanubrial placement). In 6 other dogs, the pressure sensor was placed in the upper anterior mediastinum. The animals were monitored for a minimum of 8 weeks (2 transmanubrial sensors for 12 months). Sensors that were able to maintain a midline position, high in the mediastinum, had the best signals. A caudal sensor position or abutment against an intrathoracic structure caused signal inversion (unusable signals). Transmanubrial placement resulted in a stable signal for 1 year. We conclude that long-term monitoring of respiration with a mediastinal pressure sensor can be successfully performed in dogs, providing an adequate signal for nerve-muscle stimulation. Separation from cardiovascular structures improves signal quality.

KEY WORDS — dog, long-term monitoring, mediastinal pressure sensor, respiration.

INTRODUCTION

Ideal therapies for upper airway disorders that require coordinated activation of muscles to maintain function have yet to be developed. Obstructive sleep apnea (OSA) and bilateral vocal fold paralysis are examples of such disorders. Continuous positive airway pressure (CPAP) is the most commonly used therapy for OSA,[1] yet many patients fail to continue with CPAP on a long-term basis.[2] Alternative approaches have used static positioning of the tongue base either by application of a dental appliance or by surgery. The primary deficit in OSA has been related to a decline in phasic activity of the genioglossus muscle during sleep.[3] Normal laryngeal function also requires coordination with respiration. Therapy for bilateral vocal fold paralysis typically involves destruction or impairment of normal laryngeal structures to create an acceptable airway.

Functional electrical stimulation (FES) has been used therapeutically for ventilatory insufficiency, upper and lower extremity neuromuscular-assist devices, control of intractable pain, control of incontinence, and inhibition of epileptic seizures.[4] It has also been applied to disorders of the head and neck. Stimulating the genioglossus muscle either directly or via a branch of the hypoglossal nerve can relieve pharyngeal obstruction during sleep.[5,6] In acute experiments, electrical stimulation of the hypoglossal nerve improved inspiratory airflow in animals[7] and in patients with OSA.[6] Vocal fold abduction in dogs has also been achieved with posterior cricoarytenoid (PCA) muscle stimulation, both acutely and after chronic denervation.[8,9] A small number of patients with OSA and bilateral vocal fold paralysis have been treated with FES. With further refinement, FES should provide an improvement over current therapies for OSA and bilateral vocal fold paralysis.

To prevent OSA, genioglossus muscle activity must be coordinated with the patient's attempts at respiration. Tonic activation of the genioglossus muscle would result in muscle fatigue and loss of position. Although patients can adapt to a stable cycle of vocal fold stimulation, coordination of vocal fold abduction with respiration is preferable.

The ideal respiratory signal would provide a signal that would indicate inspiration onset before inspi-

---

From the Department of Otolaryngology, University of Minnesota and Hennepin County Medical Center, Minneapolis, Minnesota (Goding), and the Department of Otolaryngology–Head and Neck Surgery (Eisele) and the Johns Hopkins Sleep Disorders Center, Division of Pulmonary and Critical Care Medicine (Schwartz), Johns Hopkins University, Baltimore, Maryland. Financial support was provided by Medtronic, Inc, Minneapolis, Minnesota. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Physiologic Research Laboratory of Medtronic, Inc.

Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

CORRESPONDENCE — George S. Goding, Jr, MD, Dept of Otolaryngology, 824 B, Hennepin County Medical Center, 701 Park Ave S, Minneapolis, MN 55415.

ratory airflow without artifact. The respiratory signal should either precede inspiration or not be delayed by more than a few tens of milliseconds. For long-term monitoring of respiration, a fully implantable device, as is used for cardiac pacing or cochlear implants, is preferable to an external device.

The current report is a compilation of 3 pilot studies that investigated the use of pressure sensors placed within the mediastinum as a means of accurately sensing the respiratory signal over a prolonged period of time. In the initial study, the pressure sensor was wired to the inner aspect of the manubrium. The second study investigated rostral and caudal orientation of the sensor tip placed loosely in the upper mediastinum. The final study examined the technique of placing the sensor through the upper manubrium.

METHODS

A total of 12 adult dogs weighing 19 to 26 kg were studied. The studies were sponsored by Medtronic, Inc, Minneapolis, Minn, and were performed at the Physiological Research Laboratories (PRL), a division of Medtronic, Inc. The studies were approved by the PRL Institutional Animal Care and Use Committee. The animals were cared for according to the US National Institutes of Health *Guide for the Care and Use of Laboratory Animals.*

ANESTHETIC TECHNIQUE

After a standard quarantine and overnight fast, the animals were sedated with 0.2 mg/kg of acepromazine maleate. Their hair was shaved in the anterior cervical and right chest regions. An intravenous catheter was placed, and approximately 20 mL of a 2.5% Pentothal solution was given. The animal was intubated, given isoflurane to effect, and mechanically ventilated. After implantation, all incisions were closed in 2 layers and a Penrose drain was placed in the neck via a separate stab incision if necessary. The animals were bandaged and allowed to recover. All drains were removed on postoperative days 2 to 4.

PRESSURE SENSOR DESCRIPTION

The pressure sensor (model 4322, Medtronic, Inc) consists of a diaphragm oriented perpendicularly to the lead body that transmits the pressure to a piezoelectric crystal. The sensor tip is 4 mm in diameter. Pressures are transmitted to the diaphragm through a hole in the silicone sensor capsule. A stainless steel sleeve 23 mm long and 5 mm in diameter was added exterior to the lead to shield the sensor from extraneous forces. The lead body is 35 cm long and ends in a connector pin that can be attached to a percutaneous threshold analyzer (PTA; Medtronic, Inc).

IMPLANTATION

Sensor Wired to Manubrium (4 Animals). After induction of anesthesia, a ventral midline incision was made in the lower part of the neck. The superior aspect of the manubrium was exposed. A pocket 1 cm in diameter and 4 cm long was created deep to the manubrium, extending into the upper mediastinum. Two holes (3 mm in diameter) were drilled in the midline of the manubrium, 3 and 5 cm from the superior edge of the manubrium. Positioning sutures were passed through the manubrial holes and brought into the neck. The positioning sutures were secured to preattached anchor wires on the pressure sensor. The positioning sutures and anchor wires were pulled through the manubrial holes, pulling the sensor into position along the deep surface of the manubrium. The anchor wires were tied along the superficial surface of the manubrium, securing the sensor in place (Fig 1A). A PTA was implanted in the lateral part of the chest wall in a subcutaneous pocket and connected to the respiratory sensor. After implantation, the cervical and thoracic incisions were closed in 2 layers.

Straight Versus J Lead (3 Animals Each). After induction of anesthesia, a ventral midline incision was made in the lower part of the neck. The superior aspect of the manubrium was exposed. A pocket 1 cm wide and 4 cm long was created deep to the manubrium, extending into the upper mediastinum. The pressure sensor was placed in one of two configurations, with the lead body anchored to the superior aspect of the manubrium with a nonabsorbable suture. Straight leads were positioned with the open (sensing) tip directed inferiorly toward the mediastinum (Fig 1B). The straight leads were anchored to the superior surface of the manubrium, 5 cm from the distal tip of the sensor. Leads with a "J" configuration (J leads) were bent back on the lead body to form the shape of a J with the open (sensing) tip directed superiorly toward the lower part of the neck (Fig 1C). Because of the J configuration, the loop of the lead body extended further into the mediastinum than the straight lead. A PTA was implanted in the lateral chest wall, in a subcutaneous pocket, and connected to the respiratory sensor. After implantation, the cervical and thoracic incisions were closed in 2 layers.

Transmanubrial Lead (2 Animals). After induction of anesthesia, a ventral midline incision was made in the lower part of the neck. The superficial surface of the manubrium was exposed. A 7-mm hole was drilled through the manubrium, 1 cm below its superior edge. The respiratory sensor was placed in the manubrial hole and secured to the perichondrium with a custom anchor. The distal (sensing) tip of the sen-

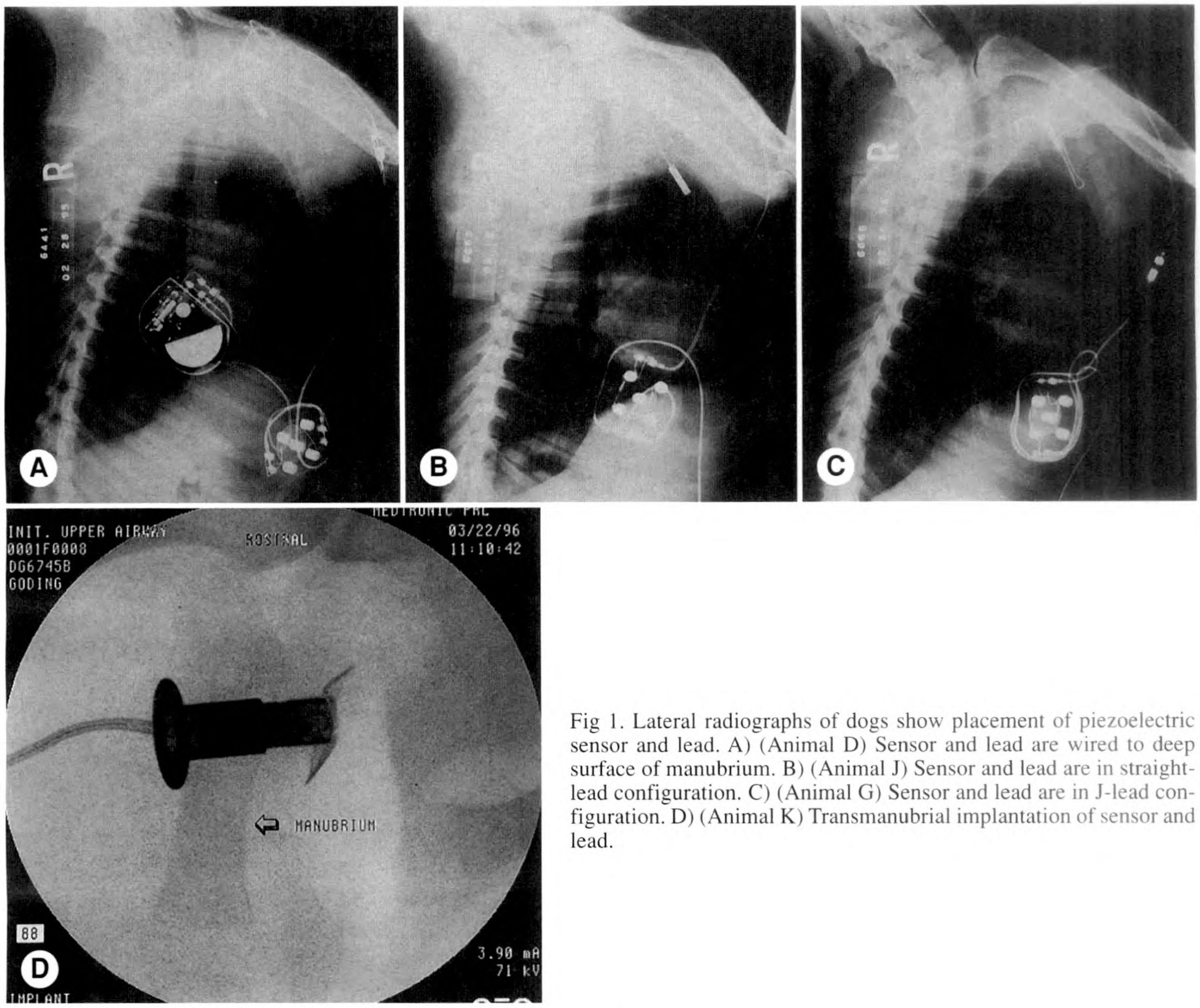


Fig 1. Lateral radiographs of dogs show placement of piezoelectric sensor and lead. A) (Animal D) Sensor and lead are wired to deep surface of manubrium. B) (Animal J) Sensor and lead are in straight-lead configuration. C) (Animal G) Sensor and lead are in J-lead configuration. D) (Animal K) Transmanubrial implantation of sensor and lead.

sor was positioned 2 to 3 mm beyond the intrathoracic manubrial surface. A 2-cm-diameter silicone "umbrella" extended from the distal sensor tip over the intrathoracic perichondrium to reduce the potential for bony overgrowth of the sensor tip (Fig 1D). The lead body was connected to an implantable pulse generator (model 2841, Medtronic, Inc) placed in the lateral part of the chest wall in a subcutaneous pocket.

DATA ACQUISITION

Sensor Wired to Manubrium. The initial monitoring was performed while the animal was breathing spontaneously during recovery from anesthesia. Percutaneous needles were placed into the input connections of the PTA and connected to a Teac 8-channel digital audiotape recorder (model RD-111, Teac, Montebello, Calif). A respiratory belt (Resp-Ez respiratory belt, EPM Systems, Midlothian, Va) was placed around the dog's chest at the level of the xiphoid process. A pneumotachograph (model 3700, Hans Rudolph, Kansas City, Mo) was connected to the endotracheal tube. Approximately 6 minutes of continuous data from the implanted respiratory sensor, respiratory belt, pneumotachograph, and electrocardiograph were recorded with the animal positioned on its sternum, back, and side (2 minutes each position). After the data recording, posterior-anterior and lateral chest radiographs were taken to confirm the sensor position.

Interim monitoring was performed 1, 2, 4, and 6 weeks after implantation, by the same procedures as the implant monitoring session, with the exception of not performing endotracheal intubation and pneumotachograph recording. The dog was sedated with 0.2 mg/kg of acepromazine maleate, with additional medication given to allow mild sedation and spontaneous ventilation. A final monitoring session was held 8 weeks after implantation, with techniques identical to those of the initial monitoring session. Posterior-anterior and lateral chest radiographs were obtained, along with fluoroscopic images, after collection of respiratory data. The animals were euthanized with a potassium chloride solution. All animals underwent

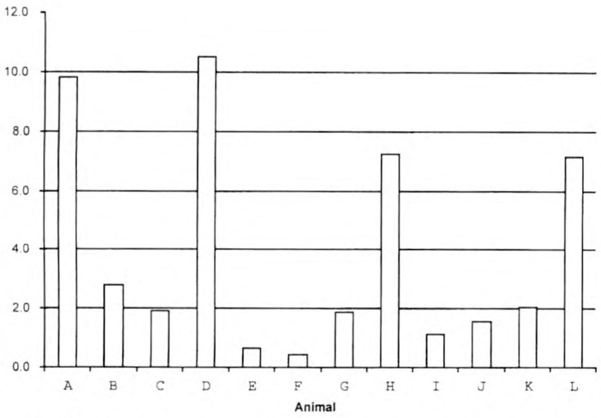


Fig 2. Signal-to-noise ratios of animals at final monitoring. Animals were lying on their side. Animals A-D — sensor wired to manubrium; animals E-G — J lead; animals H-J — straight lead; animals K and L — transmanubrial sensor.

necropsy after euthanasia.

Straight Versus J Lead. Data acquisition was performed in a similar fashion, with the exception that the final monitoring session occurred 10 to 15 weeks after implantation.

Transmanubrial Lead. Data acquisition was performed in a similar fashion, with the exception that the implantable pulse generator was linked to a personal computer–based programming system (model 3028, Medtronic, Inc). The connector pin from the respiratory sensor was surgically exposed for the final monitoring session, which occurred 1 year after implantation.

DATA ANALYSIS

A signal-to-noise (S/N) ratio was calculated from the strip chart recording of the implanted respiratory sensor, respiratory belt, electrocardiograph, and pneumotachograph (if available) from each monitoring session. The signal from the respiratory sensor was initially compared to the signal from the pneumotachograph or respiratory belt to confirm correct orientation of the respiratory signal. The respiratory signal height was divided by the cardiac artifact height for 3 representative breaths. The ratios of the 3 breaths were averaged to give an S/N ratio.

RESULTS

All animals were able to complete the study without complication. No animals demonstrated evidence of restriction of motion or respiration during the study.

SENSOR WIRED TO MANUBRIUM

In 2 animals (animals A and D), the sensor remained in the midline and was aligned with the manubrium. The S/N ratios were high in the lateral position (Fig 2) and the supine and prone positions. In animal B, the sensor was fixed, but placed at an angle, with the distal tip pointing toward the inner aspect of the manubrium. This placement resulted in an inverted respiratory signal (inadequate for stable inspiration detection) in all 3 body positions and a lower S/N ratio. Animal C was found to have a mobile sensor that had slipped lateral to the manubrium. An inverted respiratory signal was also present during quiet respiration. On fluoroscopy, the sensors in animals B and C appeared to be pushed into the manubrium (animal B) or lung (animal C) during inspiration, and these positions would potentially explain the increase in pressure. On necropsy, no major vessel or soft tissue erosion was seen. The sensor was thinly encapsulated with soft tissue, with no ingrowth of the capsule into the open sensor tip. The sensor was not adherent to any intrathoracic structures other than the manubrium.

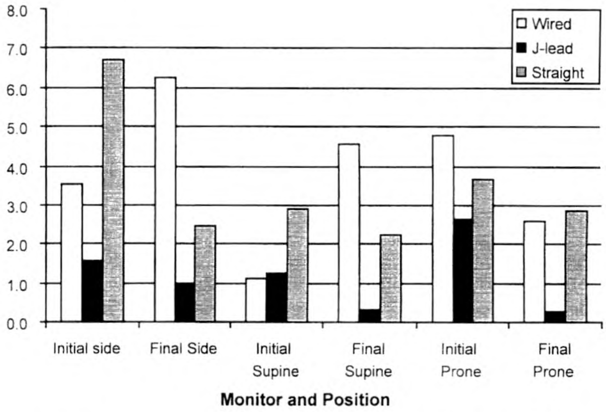


Fig 3. Average signal-to-noise ratio of wired, straight, and J-lead sensors. "Initial" and "final" refer to initial and final monitors; "prone," "supine," and "side" refer to animal position at time of reading.

J LEAD

In all 3 animals with the J lead (animals E, F, and G), a poor S/N ratio was evident. The S/N ratio was 1.0 or less in all 3 positions during the final monitoring (Fig 3). Inversion of the respiratory signal was a common finding. Because of the longer length of the J lead, it was found to lie more deeply in the mediastinum and would drift away from the manubrium into a lateral position. On necropsy, a thin capsule of fibrous tissue was present around the sensor, with no evidence of dense fibrosis or vessel erosion.

STRAIGHT LEAD

The sensors in this group showed 2 patterns of behavior. In animal H, the sensor remained in the upper mediastinum for the entire implantation period. A high S/N ratio was present in all positions (supine, 7.4; prone, 7.7; side, 7.3). In animals J and I, the initial monitor demonstrated high S/N ratios (in I:

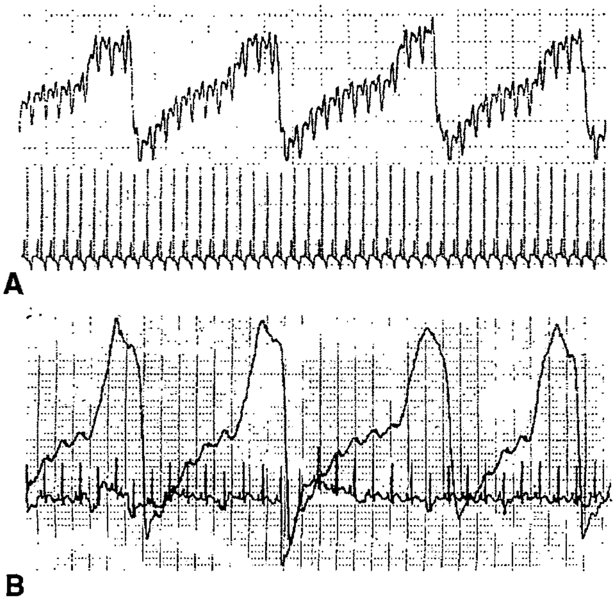


Fig 4. Respiratory signal and electrocardiographic signal, 1 year after implantation. Note that negative pressure results in positive sensor voltage output. A) (Animal K) Respiratory tracing is above electrocardiographic tracing. B) (Animal L) Signals are superimposed.

supine, 6.1; prone, 3.1; side, 4.0; in J: supine, 3.8; prone, 7.3; side, 19.7). The gradual slipping of the sensors deeper into the mediastinum, however, resulted in poor-quality respiratory signals at the final monitoring session (Fig 2). Necropsy showed thin encapsulation of the sensor tip and no vessel or soft tissue erosion by the sensor or lead body.

TRANSMANUBRIAL LEAD

The 2 animals implanted with transmanubrial sensors (animals K and L) were allowed to survive for 1 year. The respiratory waveforms for each animal are presented in Fig 4. In animal K, the S/N ratio was 2.1 at the 1-year monitoring session. The low S/N ratio was primarily due to cardiac artifact. In animal L, the S/N ratio was 7.2. On necropsy, the distal ends of the sensor tips were covered by cartilage, despite the presence of the silicone umbrella. The presence of the cartilage, however, did not adversely affect the ability of the sensor to transmit the respiratory signal.

DISCUSSION

Monitoring of respiration with a pressure sensor placed within the mediastinum of a dog has the potential to produce a usable (high S/N ratio) signal over a prolonged period of time. The common characteristics of successful implants in this study included a midline and stable position high in the mediastinum. Avoidance of signal inversion and an acceptable S/N ratio are important in allowing accurate processing of the signal and appropriate stimulation timing. If we consider an S/N ratio of 3 without signal inversion to be the threshold for an acceptable respiratory signal, 4 of the 12 dogs would have had acceptable signals. Given the diverse methods of implantation provided in this report, such a summary estimate is misleading. Caudal displacement (2 straight leads) or extension (3 J leads) was a primary cause of failure in the form of a low S/N ratio. Attempts to reduce the cardiac artifact by orienting the sensors superiorly (J leads) resulted in unsatisfactory signals. Abutment of a stable sensor tip against an intrathoracic structure was a primary cause of signal inversion in 2 leads wired to the manubrium. These problems were resolved by placing the sensor through the manubrium. An acceptable signal, however, was seen in only 1 of the 2 animals implanted in this manner for 1 year.

The anatomy of the canine thorax differs from that of the human thorax in several aspects important to this study. First, the manubrium of dogs of the size studied is cartilaginous and smaller than that of humans. It is shaped like an arrowhead pointing rostrally, rather than the thicker, flat pentagonal shape found in humans. There is no clavicular attachment to stabilize the manubrium during respiration, and the anterior chest wall is more scaphoid. The upper part of the mediastinum also contains only thin fascia separating the paired lung cavities. These differences likely result in a species discrepancy in the transmission of intrathoracic pressure at the suprasternal notch and can influence the ability to effectively sense respiration in this area. The confounding influences of cardiac artifact and tissue abutment are likely common to humans as well.

Potential methods of sensing the inspiratory phase of respiration include impedance measurement, motion measurement, monitoring of electromyographic (EMG) activity, and pressure measurement. With impedance measurement, a small current is passed between 2 electrodes. The changing dimensions of the thoracic cavity during the respiratory cycle result in varying conduction paths with varying levels of impedance. The disadvantage of impedance measurement is that signal sensitivity, noise, and wave morphology are affected by electrode placement and patient variability. All patient movement in the thoracic and/or cervical regions would affect the signal.

Tracheal elongation has been studied as a trigger for stimulation related to respiratory drive. Placement of a strain gauge along the tracheal rings was studied by Broniatowski et al.[10] The generated signal, however, is not specific to inspiration, and other actions associated with tracheal movement (deglutition and head movement) can contaminate the respiratory-related signal. Monitoring of diaphragmatic EMG activity has also been suggested.[11,12] In the dog, diaphragmatic EMG monitoring resulted in purely inspiratory signals, but was believed to be less than ideal, because of the distance from the laryngeal implant site and the long-term instability of the EMG signal. Another potential source of monitoring of respiratory activity is the phrenic nerve.[13] Cuffed electrodes around the phrenic nerve have also been investigated. Prolonged cuff recording, however, carries the risk of potential nerve damage. In a review by Zealear and Herzon,[12] monitoring of intrathoracic pressure changes was judged to have the fewest drawbacks.

The sensation of intrathoracic pressure provides a more direct measure of pulmonary mechanics than do measures of tracheal or chest wall movement. The piezoelectric sensor is stable and can function for several years. Factors other than negative-pressure inspiration may result in a pressure signal. Tissue movements relative to the sensor during respiration can also cause a sensor response. Thus, if the sensor is pushed against a solid structure during respiration, an inverse and nonfunctional signal can result. Pressure from the cardiovascular pulse transmitted through the vessels or surrounding tissues can also contaminate the signal.

Cardiac artifact was a particular problem in this study. Although many of the earlier problems reported in this series were resolved by transmanubrial placement of the sensor, excess cardiac artifact was apparent in one of the two 12-month implants. The effectiveness of monitoring respiration with mediastinal pressure sensors will likely vary with the anatomy of the individual implanted, in particular, the proximity of vascular structures to the sensors and the transmission of the pulse pressure within the mediastinum. Other options for long-term monitoring of respiration will need to be made available to treat a wide variety of patients.

ACKNOWLEDGMENTS — The authors gratefully acknowledge the technical support of Tom Senger and Ralph Bierbaum of Medtronic, Inc.

REFERENCES

1. Sullivan CE, Issa FG, Berthon-Jones M, Eves L. Reversal of obstructive sleep apnea by continuous positive airway pressure applied through the nares. Lancet 1981;1:862-5.

2. Kribbs NB, Pack AI, Kline LR, et al. Objective measurement of patterns of nasal CPAP use by patients with obstructive sleep apnea. Am Rev Respir Dis 1993;147:887-95.

3. Remmers JE, DeGroot WJ, Sauerland EK, Anch AM. Pathogenesis of upper airway occlusion during sleep. J Appl Physiol 1978;44:931-8.

4. Agnew WF, McCreery DB. Considerations for safety with chronically implanted nerve electrodes. Epilepsia 1990;31(suppl 2):S27-S32.

5. Schwartz AR, Eisele DW, Hari A, Testerman R, Erickson D, Smith PL. Electrical stimulation of the lingual musculature in obstructive sleep apnea. J Appl Physiol 1996;81:643-52.

6. Eisele DW, Smith PL, Alam DS, Schwartz AR. Direct hypoglossal nerve stimulation in obstructive sleep apnea. Arch Otolaryngol Head Neck Surg 1997;123:1-5.

7. Goding GS, Eisele DW, Testerman R, Smith PL, Roertgen K, Schwartz AR. Relief of upper airway obstruction with hypoglossal nerve stimulation in the canine. Laryngoscope 1998;108:162-9.

8. Sanders I. Electrical stimulation of laryngeal muscle. Otolaryngol Clin North Am 1991;24:1253-74.

9. Zealear DL, Rainey CL, Tanabe T, Jerles ML, Tanabe T, Herzon GD. Technical approach for reanimation of the chronically denervated larynx by means of functional electrical stimulation. Ann Otol Rhinol Laryngol 1994;103:705-12.

10. Broniatowski M, Kaneko S, Nose Y, Jacobs G, Tucker HM. Laryngeal pacemaker. II. Electronic pacing of reinnervated posterior cricoarytenoid muscles in the canine. Laryngoscope 1985;95:1194-8.

11. Otto R, Templer J, Davis W, Homeyer D, Stroble M. Coordinated electrical pacing of vocal cord abductors in recurrent laryngeal paralysis. Otolaryngol Head Neck Surg 1985;93:634-8.

12. Zealear DL, Herzon G. Progress toward the development of a chronically implantable pacemaker. In: Fujimura O, ed. Vocal physiology: voice production, mechanisms and functions. New York, NY: Raven Press, 1988:443-8.

13. Crumley RL. Experiments in laryngeal reinnervation. Laryngoscope 1982;92(suppl 30).

Ann Otol Rhinol Laryngol 110:2001

MOLECULAR MARKERS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA: THEIR BIOLOGICAL FUNCTION AND PROGNOSTIC SIGNIFICANCE

BENJAMIN D. SMITH
BRUCE G. HAFFTY, MD
CLARENCE T. SASAKI, MD
NEW HAVEN, CONNECTICUT

Head and neck squamous cell carcinoma affects more than 500,000 people worldwide each year. Despite optimal treatment with surgery, irradiation, and chemotherapy, disease recurrence and progression remains a common and challenging oncological problem. Recently, interest has developed in identifying novel molecular markers that allow identification of those patients at increased risk for locoregional recurrence and death. This article reviews several such molecular markers studied in head and neck cancer, including p53, angiogenesis-related markers, cyclin D1, and epidermal growth factor receptor. The biological function of these markers and the potential clinical implications are discussed. The purpose of this review is to update the otolaryngologist on a rapidly emerging segment of applied translational research in our field.

KEY WORDS — head and neck cancer, molecular markers, prognostic factors.

INTRODUCTION

Head and neck cancers are the sixth most prevalent cancer in the world, with a global yearly incidence of 500,000.[1] Despite advances in surgical technique and delivery of radiotherapy, the overall 5-year survival rate for patients with head and neck cancer in the United States has not improved significantly in the past 20 years, remaining at 52%.[2] Because of the high frequency of locoregional recurrence and disease progression in head and neck squamous cell carcinoma (HNSCC), interest has developed in identifying molecular markers that would enable selection of patients at risk for poor outcome. Such patients could potentially benefit from more aggressive therapy, such as elective neck dissection, hyperfractionated radiotherapy, concurrent chemotherapy, and radiosensitizing agents.[3-13] Furthermore, identification of molecular markers that influence tumor recurrence rates could lead to the development of novel pharmacotherapies targeted against specific molecular defects present in tumor cells. Our purpose in this communication is to provide a succinct review of a rapidly emerging field of translational research, in the awareness that this knowledge will continue to require updating at future intervals.

Prognostically useful molecular markers fall into several broad categories. Proto-oncogenes code for proteins that promote cellular proliferation. A proto-oncogene is transformed into an oncogene when its protein product becomes unresponsive to the normal regulatory processes that control cell division. Activation of proto-oncogenes occurs by point mutations, chromosomal translocations, or gene amplification. At the cellular level, an oncogene exerts a dominant phenotype over its proto-oncogene counterpart, because only 1 copy of an oncogene is necessary to promote neoplasia. In contrast, tumor suppressor genes (anti-oncogenes) inhibit cellular proliferation. At the cellular level, both copies of a tumor suppressor gene must be mutated to promote neoplasia. Another class of markers includes protein and growth factors that mediate the interaction between neoplastic cells and their local microenvironment. Finally, total cellular DNA content — diploid versus aneuploid — may also serve as a marker of tumor behavior.[14]

To date, a number of protein and DNA markers have been identified in HNSCC. The 4 categories of molecular markers that have received the most study are 1) p53, 2) angiogenesis-related markers, 3) cyclin D1, and 4) epidermal growth factor receptor (EGFR). Although the data remain inconclusive, these markers have potential clinical significance in determining patient prognosis and selecting appropriate therapeutic modalities. This article provides an overview of each marker studied to date, focusing on biological relevance, methods of detection, and prognostic significance. Because of the rapid proliferation of studies conducted on these topics, this article will focus primarily on studies that are particularly noteworthy because of their large sample size, originality, and/

From the Department of Therapeutic Radiology (Smith, Haffty) and the Section of Otolaryngology, Department of Surgery (Sasaki), Yale University School of Medicine, New Haven, Connecticut.

CORRESPONDENCE — Clarence T. Sasaki, MD, Section of Otolaryngology, Dept of Surgery, Yale University School of Medicine, PO Box 208041, New Haven, CT 06520.

or novel results. Although we have attempted to identify all studies that satisfy these criteria, we have undoubtedly overlooked some studies. The material presented below, however, should provide a broad and accurate review of this important and rapidly evolving literature.

P53

The tumor suppressor gene p53 negatively regulates the cell cycle and serves to protect the integrity of the genome. In response to certain types of DNA damage, p53 protein levels increase, resulting in G1 arrest or apoptosis.[15-18] Interestingly, p53 is required for chemotherapy- and radiation-induced apoptosis. Thus, p53 mutant cells exhibit more resistance to ionizing radiation and certain chemotherapeutic agents than wild-type p53 cells.[19-21] This evidence led to the hypothesis that tumors lacking a functional p53 gene will be radioresistant because of a deficiency in radiation-induced apoptosis.[22] This hypothesis has been strengthened by experiments conducted on a p53 mutant cell line derived from a human HNSCC. Transfection of this cell line with an adenoviral vector containing a functional p53 gene resulted in a dose-dependent increase in radiation sensitivity and restoration of the G1 checkpoint and apoptotic pathway.[23,24] It may also be that p53 mediates hypoxia-induced apoptosis. Graeber et al[25] demonstrated that mutations in p53 reduce hypoxic cell death and confer a competitive advantage to cells growing in a hypoxic environment. Thus, mutations in p53 may increase the number of hypoxic cells, conferring hypoxia-mediated radioresistance. As a whole, the data from basic science studies support the hypothesis that p53 mutations contribute to biologically aggressive tumor behavior by conferring resistance to radiotherapy and chemotherapy.

In light of this evidence, numerous groups have examined the prognostic significance of p53 mutations in HNSCC cohorts. Many studies have detected p53 mutations by means of immunohistochemistry (IHC), capitalizing on the differential expression of wild-type and mutant p53. Ordinarily, wild-type p53 is not present at a high enough concentration to be detected by IHC, whereas most p53 mutations confer stabilization to the protein and result in high intracellular levels.[26,27] In addition to IHC, some groups have used polymerase chain reaction–single strand conformation polymorphism analysis to screen for mutations in commonly mutated exons of the p53 gene. Finally, at least 1 group has directly detected p53 mutations by amplifying and sequencing exons in the conserved region of the p53 gene.[28]

Results of studies evaluating the prognostic significance of p53 remain mixed; however, several excellent studies have demonstrated an association between p53 mutation and poor outcome in HNSCC. Koch et al[28] evaluated the prognostic significance of p53 mutations by amplifying and directly sequencing exons 5 through 9 in 110 primary HNSCCs treated with either primary or adjuvant radiotherapy. These tumors spanned all major sites for primary HNSCC and included all clinical stages. Mutations of p53 resulted in a statistically significant increased risk for locoregional recurrence, but did not correlate with the overall survival rate. Several other studies that detected p53 abnormalities by IHC have also reported a significant relationship between p53 mutation and poor outcome. Shin et al[29] found p53 overexpression to correlate with tumor recurrence, a poor disease-free survival rate, and a poor overall survival rate in 69 patients with T1-4N0-3 HNSCCs treated with surgery and/or radiotherapy. In a group of 106 patients with stage II to IV oropharyngeal squamous cell carcinoma (SCC) treated with surgery with or without adjuvant radiotherapy, Caminero et al[30] found p53 overexpression to predict poor local control and a poor overall survival rate. In addition, Raybaud-Diogene et al[31] found p53 overexpression to predict poor rates of local control, disease-free survival, and overall survival in 98 patients with HNSCCs treated with radiotherapy with or without neck dissection. Finally, Narayana et al[32] reported a correlation between p53 overexpression and local recurrence in 102 T1N0M0 glottic SCCs treated with radiotherapy. Taken as a whole, these studies provide convincing data on the role of p53 mutations in predicting poor locoregional control and the likely role of p53 mutations in predicting a poor survival rate.

Despite these promising results, several other studies conducted on large cohorts have failed to find a prognostic role for p53 mutations detected with IHC. In 115 T1-4N0-3 HNSCCs treated with surgery with or without postoperative radiotherapy, Michalides et al[33] failed to uncover a relationship between p53 overexpression and disease-free survival. Gluckman et al[34] reported no significant relationship between p53 overexpression and locoregional control in 51 patients with T1 SCC of the ventral tongue or floor of the mouth who were treated with surgery alone. In another cohort of patients managed exclusively with surgery, Veneroni et al[35] reported no association between p53 overexpression and overall survival in 69 patients with T2-4 oral or oropharyngeal SCC. Finally, Pai et al[36] failed to find an association between p53 overexpression and local control or disease-free survival in a cohort of 86 T1-2 glottic SCCs treated with primary radiotherapy. Notably, with the exception of the Pai et al study, all of the "negative" studies were conducted on cohorts in which a large fraction, from 36% to 100%, of the patients were treated with surgery alone. In contrast, all of the studies that showed an association between p53 abnormality and prognosis were conducted on cohorts in which a small fraction, from 0% to 31%, of the patients were treated with surgery alone. These observations, consistent with the laboratory data, suggest that p53 status may aid in the determination of prognosis for patients treated with either primary or adjuvant radiotherapy.

ANGIOGENESIS-RELATED MARKERS

Angiogenesis, the sprouting of new blood vessels from a preexisting endothelium, enables the growth of tumors beyond microscopic size.[37,38] Angiogenic activity can be assessed in archival tumor tissue directly, by counting the density of microvessels perfusing a tumor, or indirectly, by staining with antibodies directed against angiogenesis-promoting factors. Many growth factors and cytokines, including vascular endothelial growth factor (VEGF), have been shown to promote angiogenesis.[39] A 34 to 50 kd dimer composed of 2 identical disulfide-linked subunits that arise from differential splicing of a single gene,[40] VEGF is induced under hypoxic conditions and stimulates endothelial cell proliferation and vascular permeability by binding to its receptor tyrosine kinases Flt-1 and KDR/FLK-1.[41-45] Because hypoxic tumors display increased radioresistance, VEGF protein levels may serve as a surrogate marker for hypoxic radioresistance. Furthermore, angiogenic activity may influence a tumor's metastatic potential by exposing it to a greater endothelial surface area, thus increasing the likelihood of hematogenous dissemination.[46] In addition, once a tumor has formed a distant micrometastasis, it must be able to recruit a vascular supply in order to proliferate to a clinically relevant size.

A number of studies have attempted to assess the extent of tumor angiogenic activity by means of IHC staining of microvessels. By means of antibodies directed to markers that are found specifically on endothelial cells, such as factor VIII, CD-31, and CD-34, the density of microvessels is counted and averaged. Williams et al[47] reported a positive relationship between tumor microvessel density (MVD) and regional relapse in 66 T1-3N0 oral SCCs treated with surgical excision with or without neck dissection. These results have been confirmed in 3 other series of oral SCC patients treated with surgery.[48-50] In contrast, Dray et al[51] failed to find an association between tumor MVD and metastatic disease, local recurrence, disease-free survival, or overall survival in 106 T1-3N0M0 HNSCCs treated with surgery and/or radiotherapy. Further, Salven et al[52] showed that tumor MVD did not predict regional recurrence or overall survival in 156 stage I to IV HNSCCs treated with radical surgery and postoperative radiotherapy. Taken together, these studies show some potential for tumor MVD as a predictor of regional metastatic disease in early-stage oral SCC treated with surgery alone. However, the generalizability of these data to other sites in the head and neck treated with other therapeutic modalities is questionable. Furthermore, tumor MVD has not been shown to predict either local recurrence or survival.

Directly assessing expression of angiogenic factors offers another approach to the study of tumor angiogenesis. Denhart et al[53] demonstrated that VEGF messenger RNA (mRNA) and its receptor's mRNA are strongly expressed in most HNSCCs. Maeda et al[54] measured tumor VEGF levels with IHC in a cohort of 45 stage I to IV oral SCCs treated with radiotherapy alone or with preoperative radiotherapy plus resection. This study showed a significant relationship between VEGF expression and a poor overall survival rate. In an additional study conducted on 36 patients who had undergone primary surgical resection for HNSCC, high VEGF levels detected with enzyme-linked immunosorbent assay correlated with tumor recurrence and a shortened disease-free interval.[55] In an unpublished study conducted in our laboratory on 56 stage II to IV oral and oropharyngeal SCCs treated with surgical excision and postoperative radiotherapy, VEGF expression predicted poor local control and increased risk for distant metastatic disease. Furthermore, VEGF expression was an independent predictor of poor disease-free and overall survival rates. Despite these promising results, Salven et al[52] failed to detect a significant relationship between VEGF staining and nodal metastasis or overall survival in their cohort of 156 stage I to IV HNSCCs treated with radical surgery and postoperative radiotherapy. Given these conflicting results, the ultimate prognostic value of VEGF expression in HNSCC remains unclear. However, sufficient evidence exists to justify further studies with larger cohorts in order to elucidate the precise relationship between VEGF expression and locoregional control, distant metastasis, and survival.

CYCLIN D1

Cyclin D1, also known as PRAD1, is a proto-oncogene located on chromosome 11q13.[56] Cyclin D1 responds to extracellular mitogens and is a rate-limiting controller of G1-phase progression through the cell cycle.[57] Complexes of cyclin D1 and the cyclin-dependent kinases cdk4 or cdk6 mediate progression through G1 by phosphorylating pRB. Overexpression of cyclin D1 shortens the G1 interval and reduces the cell's dependence on mitogens for proliferation.

For example, Zwijsen et al[58] found that overexpression of cyclin D1 in a human epithelial breast cancer cell line reduced the fraction of cells entering quiescence under conditions in which growth factors were limited, resulting in accelerated growth and hyperproliferation. Such observations suggest that deregulation of cyclin D1 may increase the overall aggressiveness of certain cancers by desensitizing cellular proliferation to inhibitory signals.

In HNSCC, deregulation of cyclin D1 has been measured by a variety of methods. At the protein level, several studies have assessed cyclin D1 expression by IHC. At the DNA level, a number of techniques, including cytogenetics, Southern blot analysis, polymerase chain reaction gene amplification, and fluorescent in situ hybridization, have been used to measure cyclin D1 gene amplification.[59]

Although the exact prognostic significance of cyclin D1 deregulation has not been completely determined, current evidence suggests a relationship between cyclin D1 amplification and poor prognosis. Michalides et al[33] examined a cohort of 115 T1-4N0-3 HNSCC patients treated with surgery with or without postoperative radiotherapy. Cyclin D1 protein levels predicted tumor recurrence and a poor disease-free survival rate. Similarly, Pignataro et al[60] selected a cohort of 149 stage I to IV laryngeal SCCs treated with surgery with or without postoperative radiotherapy. Cyclin D1 overexpression correlated with an advanced T stage, nodal metastases, a poor disease-free survival rate, and a poor overall survival rate in bivariate analysis. In addition to these studies, 5 other studies conducted on smaller cohorts of 45 to 75 patients have shown a relationship between cyclin D1 amplification and a poor prognosis in HNSCC.[61-65] In contrast, Muller et al[66] failed to find a relationship between 11q13 amplification and disease-free or overall survival in 280 T1-4N0-3 HNSCCs treated with either surgery alone (79 patients) or surgery and postoperative radiotherapy (201 patients). Only 1 other study, conducted on 50 patients, failed to correlate cyclin D1 amplification with outcome.[67] Finally, Yoo et al[68] found high cyclin D1 protein expression to be protective against local recurrence in a case control study of 60 T1-2N0 laryngeal SCCs treated only with radiotherapy. Nevertheless, the cumulative weight of evidence implicates cyclin D1 deregulation as an important determinant of poor prognosis in most stages and sites of HNSCC.

In addition to the relationship between cyclin D1 amplification and poor prognosis, a specific A/G polymorphism that modulates splicing of cyclin D1 mRNA may also influence prognosis.[69] Matthias et al[70] examined 384 patients with stage I to IV HNSCCs treated with surgery and other appropriate modalities. The cyclin D1 genotype did not influence susceptibility to HNSCC and did not correlate with T stage, N stage, patient age, or gender. Homozygosity for the G allele, the GG genotype, was associated with poorly differentiated tumors. When the investigators controlled for the relationship between genotype and differentiation, the GG genotype correlated with a poor disease-free survival rate in laryngeal and pharyngeal tumors. Future investigations along this avenue of research should be conducted to determine the relationship between this genotype and cyclin D1 function within malignant cells.

EPIDERMAL GROWTH FACTOR RECEPTOR

Epidermal growth factor receptor and its ligand transforming growth factor–α (TGF-α) are frequently overexpressed in HNSCC.[71,72] As a receptor tyrosine kinase, EGFR activation induces autophosphorylation, resulting in downstream activation of the protooncogene Ras.[73] Consistent with its downstream cell signaling pathways, activation of EGFR has been shown to promote cellular proliferation. In tumor-derived cell lines, both inhibition of TGF-α with antisense oligonucleotides and inhibition of EGFR with antisense oligonucleotides, monoclonal antibodies, or specific inhibitors of EGFR kinase activity resulted in reduced cellular proliferation.[74,75] These observations have led to the hypothesis that autocrine activation of EGFR by TGF-α promotes tumor growth in HNSCC. In addition, expression of EGFR may modulate tumor radiosensitivity. Huang et al[76] showed that in cell lines derived from HNSCC patients, blockade of the EGF receptor with the C225 monoclonal antibody resulted in growth inhibition, accumulation of cells in G1 phase, induction of apoptosis, enhancement of radiosensitivity, and amplification of radiation-induced apoptosis. In other SCC cell lines, however, EGFR activation has been shown to enhance radiosensitivity.[77-79] Hence, the relationship of EGFR activation to radiosensitivity may vary according to the precise downstream signaling components present in specific neoplastic cell lines.

In agreement with laboratory data, clinical studies have established a relationship between EGFR and TGF-α expression and poor prognosis. Initial evidence for the prognostic utility of EGFR levels was provided by Dassonville et al,[80] who examined EGFR levels using a quantitative radioligand receptor assay (RRA) in 109 stage I to IV HNSCC patients treated primarily with either chemotherapy or surgery. Expression of EGFR significantly predicted tumor recurrence and a poor disease-free survival rate. Maurizi et al[81] used an RRA to establish a relationship between EGFR expression and tumor recurrence and a poor disease-free survival rate in 140 T1-4N0-3 laryngeal SCCs treated primarily with surgery. Multivariate analysis revealed that EGFR levels were an important determinant of cervical node metastasis, with 5-year metastasis-free survival rates of 66% for EGFR-negative tumors and 15% for EGFR-positive tumors.[82] Similarly, Grandis et al[83] used quantitative IHC to show that increasing levels of both TGF-α and EGFR predicted a poor disease-free survival rate and a poor cause-specific survival rate in a cohort of 91 patients with T1-4N0-2 HNSCCs. All of the patients in this cohort were treated with surgery, and 56 received postoperative radiotherapy. Finally, Wen et al[84] reported a study of 68 stage I or II laryngeal SCCs treated with radiotherapy alone. On qualitative IHC, TGF-α levels predicted tumor recurrence but did not correlate with the overall survival rate. Expression of EGFR was not predictive of tumor recurrence or survival. The failure of the Wen et al study to concur with other studies could simply be attributable to a lack of statistical power or to deficiencies inherent in qualitative IHC. However, because this was the only study conducted on a cohort uniformly treated with radiotherapy, it may be that EGFR expression fails to modulate radiosensitivity in early-stage laryngeal SCC. Thus, the cumulative weight of evidence from clinical studies supports a role for EGFR and TGF-α protein levels in determining a poor prognosis for patients treated with surgery or chemotherapy. However, the precise relationship between EGFR expression and radiosensitivity merits further research in both basic science and clinical settings.

CONCLUSIONS

The identification of novel molecular markers and the evaluation of their prognostic significance represents an exciting advancement in head and neck oncology. Despite some conflicting results, evidence is mounting for the potential prognostic significance of p53, angiogenesis-related markers, cyclin D1, and EGFR in the treatment of HNSCC. With continued efforts to establish homogeneous study populations, standardize methods for marker detection, and increase statistical power, the precise predictive ability of various molecular markers for all tumor sites, stages, and treatment modalities should become increasingly clear. In the future, knowledge of tumor aggressiveness at the molecular level may be integrated with standard clinicopathological parameters in the staging of head and neck cancer. Further, the ability to detect molecular lesions in HNSCC may assist in tumor staging by enabling the molecular diagnosis of histologically occult disease.[85] These advancements would promote more appropriate selection of therapeutic modalities, thereby minimizing the morbidity associated with unnecessary treatments and maximizing the potential for complete tumor eradication.

In addition to their prognostic value, molecular markers serve as optimal targets for the development of new cancer pharmacotherapies. Already, laboratory experiments have demonstrated potential for inhibiting tumor growth by replacing mutated tumor suppressor genes such as p53 and p16 with their wild-type alleles.[86,87] In addition, proto-oncogenes that reside on the cell surface, such as EGFR, serve as optimal targets for monoclonal antibody therapy. Phase I studies evaluating the efficacy of C225, a monoclonal antibody specific for EGFR, have shown potential for a clinically significant benefit in using C225 as a potentiator of radiotherapy and certain chemotherapeutic agents.[88,89] In contrast to surgery and radiotherapy, these new therapies directly inhibit the molecular machinery that promotes malignancy, thereby offering a new approach to cancer treatment that may increase cure rates and decrease deleterious side effects. Continued efforts to identify molecular markers, determine their prognostic significance, and develop appropriate pharmacological interventions will likely result in substantial improvements in the management of patients with HNSCC.

REFERENCES

1. Parkin DM, Laara E, Muir CS. Estimates of the worldwide frequency of sixteen major cancers in 1980. Int J Cancer 1988;41:184-97.

2. Swango PA. Cancers of the oral cavity and pharynx in the United States: an epidemiologic overview. J Public Health Dent 1996;56:309-18.

3. Haffty BG. Concurrent chemoradiation in the treatment of head and neck cancer. Hematol Oncol Clin North Am 1999; 13:719-42.

4. Brizel DM, Albers ME, Fisher SR, et al. Hyperfractionated irradiation with or without concurrent chemotherapy of locally advanced head and neck cancer. N Engl J Med 1998;338: 1798-803.

5. Overgaard J. Clinical evaluation of nitroimidazoles as modifiers of hypoxia in solid tumors. Oncol Res 1994;6:509-18.

6. Haffty B, Son YH, Papac R, et al. Chemotherapy as an adjunct to radiation in the treatment of squamous cell carcinoma of the head and neck: results of the Yale mitomycin randomized trials. J Clin Oncol 1997;15:268-76.

7. Rockwell S. Oxygen delivery: implications for the biology and therapy of solid tumors. Oncol Res 1997;9:383-90.

8. Horiot JC, Bontemps P, van den Bogaert W, et al. Accelerated fractionation (AF) compared to conventional fractionation (CF) improves loco-regional control in the radiotherapy of advanced head and neck cancers: results of the EORTC 22851

randomized trial. Radiother Oncol 1997;44:111-21.

9. Munro AJ. An overview of randomised controlled trials of adjuvant chemotherapy in head and neck cancer. Br J Cancer 1995;71:83-91.

10. Mauceri HJ, Hann NN, Beckett MA, et al. Combined effects of angiostatin and ionizing radiation in antitumour therapy. Nature 1998;394:287-91.

11. Vokes EE, Athanasiadia I. Chemotherapy for squamous cell carcinoma of the head and neck: the future is now. Ann Oncol 1996;7:15-29.

12. Brown JM, Siim BG. Hypoxia-specific cytotoxins in cancer therapy. Semin Radiat Oncol 1996;6:22-36.

13. Forastiere AA, Urba SG. Single-agent paclitaxel plus ifosfamide in the treatment of head and neck cancer. Semin Oncol 1995;22:24-7.

14. Stell PM. Ploidy in head and neck cancer: a review and meta-analysis. Clin Otolaryngol 1991;16:510-6.

15. Lane DP. p53, Guardian of the genome. Nature 1992;358: 15-6.

16. Lane DP. The regulation of p53 function: Steiner Award lecture. Int J Cancer 1994;57:623-7.

17. Hunter T. Braking the cycle. Cell 1993;75:839-41.

18. Yonish-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M. Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature 1991; 352:345-7.

19. Clarke AR, Purdie CA, Harrison DJ, et al. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature 1993;362:849-52.

20. Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T. p53 Is required for radiation-induced apoptosis in mouse thymocytes. Nature 1993;362:847-9.

21. Lowe SW, Ruley HE, Jacks T, Housman DE. p53-Dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 1993;74:957-67.

22. Blank KR, Rudoltz MS, Kao GD, Muschell RJ, McKenna WG. The molecular regulation of apoptosis and implications for radiation oncology. Int J Radiat Biol 1997;71:455-66.

23. Chang EH, Jang YJ, Hao Z, et al. Restoration of the G1 checkpoint and the apoptotic pathway mediated by wild-type p53 sensitizes squamous cell carcinoma of the head and neck to radiotherapy. Arch Otolaryngol Head Neck Surg 1997;123: 507-12.

24. Pirollo KF, Hao Z, Rait A, et al. p53 Mediated sensitization of squamous cell carcinoma of the head and neck to radiotherapy. Oncogene 1997;14:1735-46.

25. Graeber TG, Osmanian C, Jacks T, et al. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumors. Nature 1996;379:88-91.

26. Jenkins JR, Rudge K, Chumakov P, Currie GA. The cellular oncogene p53 can be activated by mutagenesis. Nature 1985; 317:816-8.

27. Iggo R, Gatter K, Bartek J, Lane D, Harris AL. Increased expression of mutant forms of p53 oncogene in primary lung cancer. Lancet 1990;335:675-9.

28. Koch WM, Brennan JA, Zahurak M, et al. p53 Mutation and locoregional treatment failure in head and neck squamous cell carcinoma. J Natl Cancer Inst 1996;88:1580-6.

29. Shin DM, Lee JS, Lippman SM, et al. p53 Expression: predicting recurrence and second primary tumors in head and neck squamous cell carcinoma. J Natl Cancer Inst 1996;88:519-29.

30. Caminero MJ, Nunez F, Suarez C, Ablanedo P, Riera JR, Dominguez F. Detection of p53 protein in oropharyngeal carcinoma. Prognostic implications. Arch Otolaryngol Head Neck Surg 1996;122:769-72.

31. Raybaud-Diogene H, Fortin A, Morency R, Roy J, Monteil RA, Tetu B. Markers of radioresistance in squamous cell carcinomas of the head and neck: a clinicopathologic and immunohistochemical study. J Clin Oncol 1997;15:1030-8.

32. Narayana A, Vaughan AT, Kathuria S, Fisher SG, Walter SA, Reddy SP. p53 Overexpression is associated with bulky tumor and poor local control in T1 glottic cancer [Abstract]. Int J Radiat Oncol Biol Phys 1998;42(suppl):146.

33. Michalides RJ, van Veelen NM, Kristel PM, et al. Overexpression of cyclin D1 indicates a poor prognosis in squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg 1997;123:497-502.

34. Gluckman JL, Pavelic ZP, Welkoborsky HJ, et al. Prognostic indicators for squamous cell carcinoma of the oral cavity: a clinicopathologic correlation. Laryngoscope 1997;107: 1239-44.

35. Veneroni S, Silvestrani R, Costa A, Salvatori P, Faranda A, Molinari R. Biological indicators of survival in patients treated by surgery for squamous cell carcinoma of the oral cavity and oropharynx. Oral Oncol 1997;33:408-13.

36. Pai HH, Rochon L, Clark B, Black M, Shenouda G. Overexpression of p53 protein does not predict local-regional control or survival in patients with early-stage squamous cell carcinoma of the glottic larynx treated with radiotherapy. Int J Radiat Oncol Biol Phys 1998;41:37-42.

37. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971;285:1182-6.

38. Folkman J. Tumor angiogenesis. Adv Cancer Res 1985; 43:175-203.

39. Klagsbrun M, D'Amore PA. Regulators of angiogenesis. Annu Rev Physiol 1991;53:217-39.

40. Klagsbrun M, Soker S. VEGF/VPF: the angiogenesis factor found? Curr Biol 1993;3:699-702.

41. Plate KH, Breier G, Weich HA, Risau W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 1992;359:845-8.

42. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1992;359:843-5.

43. Shweiki D, Neeman M, Itin A, Keshet E. Induction of vascular endothelial growth factor expression by hypoxia and by glucose deficiency in multicell spheroids: implications for tumor angiogenesis. Proc Natl Acad Sci U S A 1995;92:768-72.

44. Hlatky L, Tsionou C, Hahnfeldt P, Coleman CN. Mammary fibroblasts may influence breast tumor angiogenesis via hypoxia-induced vascular endothelial growth factor up-regulation and protein expression. Cancer Res 1994;54:6083-6.

45. Kroll J, Waltenberger J. The vascular endothelial growth factor receptor KDR activates multiple signal transduction pathways in porcine aortic endothelial cells. J Biol Chem 1997;272: 32521-7.

46. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis — correlation in invasive breast carcinoma. N Engl J Med 1991;324:1-8.

47. Williams JK, Carlson GW, Cohen C, Derose PB, Hunter S, Jurkiewicz MJ. Tumor angiogenesis as a prognostic factor in oral cavity tumors. Am J Surg 1994;168:373-80.

48. Alcalde RE, Shintani S, Yoshihama Y, Matsumara T. Cell proliferation and tumor angiogenesis in oral squamous cell carcinoma. Anticancer Res 1995;15:1417-22.

49. Shpitzer T, Chaimoff M, Gal R, Stern Y, Feinmesser R, Segal K. Tumor angiogenesis as a prognostic factor in early oral tongue cancer. Arch Otolaryngol Head Neck Surg 1996;122: 865-8.

50. Alcalde RE, Terakado N, Otsuki K, Matsumara T. Angiogenesis and expression of platelet-derived endothelial cell growth factor in oral squamous cell carcinoma. Oncology 1997; 54:324-8.

51. Dray TG, Hardin NJ, Sofferman RA. Angiogenesis as a prognostic marker in early head and neck cancer. Ann Otol Rhinol Laryngol 1995;104:724-9.

52. Salven P, Heikkila P, Anttonen A, Kajanti M, Joensuu H. Vascular endothelial growth factor in squamous cell head and neck carcinoma: expression and prognostic significance. Mod Pathol 1997;10:1128-33.

53. Denhart BC, Guidi AJ, Tognazzi K, Dvorak HF, Brown LF. Vascular permeability factor/vascular endothelial growth factor and its receptors in oral and laryngeal squamous cell carcinoma and dysplasia. Lab Invest 1997;77:659-64.

54. Maeda T, Matsumura S, Hiranuma H, et al. Expression of vascular endothelial growth factor in human oral squamous cell carcinoma: its association with tumour progression and p53 gene status. J Clin Pathol 1998;51:771-5.

55. Eisma RJ, Spiro JD, Kreutzer DL. Vascular endothelial growth factor expression in head and neck squamous cell carcinoma. Am J Surg 1997;174:513-7.

56. Motokura T, Bloom T, Kim HG, et al. A novel cyclin encoded by a bcl1-linked candidate oncogene. Nature 1991;350: 512-5.

57. Sherr CJ. D-type cyclins. Trends Biochem Sci 1995;20: 187-90.

58. Zwijsen RM, Klompmaker R, Wientjens EB, Kristel PM, van der Burg B, Michalides RJ. Cyclin D1 triggers autonomous growth of breast cancer cells by governing cell cycle exit. Mol Cell Biol 1996;16:2554-60.

59. Alavi S, Namazie A, Calcaterra TC, Wang TC, Srivatsan ES. Clinical application of fluorescence in situ hybridization for chromosome 11q13 analysis in head and neck cancer. Laryngoscope 1999;109:874-9.

60. Pignataro L, Pruneri G, Carboni N, et al. Clinical relevance of cyclin D1 protein overexpression in laryngeal squamous cell carcinoma. J Clin Oncol 1998;16:3069-77.

61. Meredith SD, Levine PA, Burns JA, et al. Chromosome 11q13 amplification in head and neck squamous cell carcinoma. association with poor prognosis. Arch Otolaryngol Head Neck Surg 1995;121:790-4.

62. Bellacosa A, Almadori G, Cavallo S, et al. Cyclin D1 gene amplification in human laryngeal squamous cell carcinomas: prognostic significance and clinical implications. Clin Cancer Res 1996;2:175-80.

63. Akervall JA, Michalides RJ, Mineta H, et al. Amplification of cyclin D1 in squamous cell carcinoma of the head and neck and the prognostic value of chromosomal abnormalities and cyclin D1 overexpression. Cancer 1997;79:380-9.

64. Kyomoto R, Kumazawa H, Toda Y, et al. Cyclin-D1-gene amplification is a more potent prognostic factor than its protein over-expression in human head-and-neck squamous-cell carcinoma. Int J Cancer 1997;74:576-81.

65. Nogueira CP, Dolan RW, Gooey J, et al. Inactivation of p53 and amplification of cyclin D1 correlate with clinical outcome in head and neck cancer. Laryngoscope 1998;108:345-50.

66. Muller D, Millon R, Velten M, et al. Amplification of 11q13 DNA markers in head and neck squamous cell carcinomas: correlation with clinical outcome. Eur J Cancer 1997;33: 2203-10.

67. Fortin A, Guerry M, Guerry R, et al. Chromosome 11q13 gene amplifications in oral and oropharyngeal carcinomas: no correlation with subclinical lymph node invasion and disease recurrence. Clin Cancer Res 1997;3:1609-14.

68. Yoo S, Carter D, Turner B, et al. Prognostic significance of cyclin D1 protein levels in early stage larynx cancer treated with primary radiation. Int J Cancer Radiat Oncol Invest 2000; 90:22-8.

69. Betticher D, Thatcher N, Altermatt HJ, Hoban P, Ryder WD, Heighway J. Alternate splicing produces a novel cyclin D1 transcript. Oncogene 1995;11:1005-11.

70. Matthias C, Branigan K, Jahnke V, et al. Polymorphism within the cyclin D1 gene is associated with prognosis in patients with squamous cell carcinoma of the head and neck. Clin Cancer Res 1998;4:2411-8.

71. Grandis JR, Tweardy DJ. Elevated levels of transforming growth factor-alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res 1993;53:3579-84.

72. Grandis JR, Melhem MF, Barnes EL, Tweardy DJ. Quantitative immunohistochemical analysis of transforming growth factor-alpha and epidermal growth factor receptor in patients with squamous cell carcinoma of the head and neck. Cancer 1996;78:1284-92.

73. Hackel PO, Zwick E, Prenzel N, Ullrich A. Epidermal growth factor receptors: critical mediators of multiple receptor pathways. Curr Opin Cell Biol 1999;11:184-9.

74. Grandis JR, Chakraborty A, Zeng Q, Melhem MF, Tweardy DJ. Downmodulation of TGF-alpha protein expression with antisense oligonucleotides inhibits proliferation of head and neck squamous carcinoma but not normal mucosal epithelial cells. J Cell Biochem 1998;69:55-62.

75. Rubin Grandis J, Chakraborty A, Melhem MF, Zeng Q, Tweardy DJ. Inhibition of epidermal growth factor receptor gene expression and function decreases proliferation of head and neck squamous carcinoma but not normal mucosal epithelial cells. Oncogene 1997;15:409-16.

76. Huang SM, Bock JM, Harari PM. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. Cancer Res 1999;59:1935-40.

77. Kwok TT, Sutherland RM. Enhancement of sensitivity of human squamous carcinoma cells to radiation by epidermal growth factor. J Natl Cancer Inst 1989;81:1020-4.

78. Kwok TT, Sutherland RM. Differences in EGF related radiosensitization of human squamous carcinoma cells with high

and low numbers of EGF receptors. Br J Cancer 1991;64:251-4.

79. Bonner JA, Maihle NJ, Folven BR, Christianson TJ, Spain K. The interaction of epidermal growth factor and radiation in human head and neck squamous cell carcinoma cell lines with vastly different radiosensitivities. Int J Radiat Oncol Biol Phys 1994;29:243-7.

80. Dassonville O, Formenta J, Francoual M, et al. Expression of epidermal growth factor receptor and survival in upper aerodigestive tract cancer. J Clin Oncol 1993;11:1873-8.

81. Maurizi M, Almadori G, Ferrandina G, et al. Prognostic significance of epidermal growth factor receptor in laryngeal squamous cell carcinoma. Br J Cancer 1996;74:1253-7.

82. Almadori G, Cadoni G, Galli J, et al. Epidermal growth factor receptor expression in primary laryngeal cancer: an independent prognostic factor of neck node relapse. Int J Cancer 1999;84:188-91.

83. Grandis JR, Melhem M, Gooding W, et al. Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. J Natl Cancer Inst 1998;90:824-32.

84. Wen QH, Miwa T, Yoshizaki T, Nagayama I, Furukawa M, Nishijima H. Prognostic value of EGFR and TGF-alpha in early laryngeal cancer treated with radiotherapy. Laryngoscope 1996;106:884-5.

85. Brennan JA, Sidransky D. Molecular staging of head and neck squamous carcinoma. Cancer Metastasis Rev 1996;15:3-10.

86. Clayman GL, Liu TJ, Overholt SM, et al. Gene therapy for head and neck cancer: comparing the tumor suppressor gene p53 and a cell cycle regulator WAF1/CIP1 (p21). Arch Otolaryngol Head Neck Surg 1996;122:489-93.

87. Rocco JW, Li D, Liggett WH, et al. p16INK4A adenovirus-mediated gene therapy for human head and neck squamous cell cancer. Clin Cancer Res 1998;4:1697-704.

88. Mendelsohn J. Epidermal growth factor receptor inhibition by a monoclonal antibody as anticancer therapy. Clin Cancer Res 1997;3:2703-7.

89. Wheeler RH, Spencer S, Buchsbaum D, Robert F. Monoclonal antibodies as potentiators of radiotherapy and chemotherapy in the management of head and neck cancer. Curr Opin Oncol 1999;11:187-90.

Ann Otol Rhinol Laryngol 110:2001

OBJECTIVE VOICE ANALYSIS AFTER AUTOLOGOUS FAT INJECTION FOR UNILATERAL VOCAL FOLD PARALYSIS

DANA M. HARTL, MD STÉPHANE HANS, MD JACQUELINE VAISSIÈRE, PHD
MARC RIQUET, MD OLLIVIER LACCOURREYE, MD DANIEL F. BRASNU, MD
PARIS, FRANCE

This study was designed to objectively compare a patient's voice after onset of unilateral vocal fold paralysis (UVFP) to his or her own normal voice, and to compare the results after treatment by intrafold injection of autologous fat. Acoustic recordings were obtained for 2 male patients before thoracic surgery and after the onset of iatrogenic left UVFP. Vocal fold augmentation was performed 10 days after UVFP. The acoustic recordings were repeated within 3 days and at 1 month. The phonation quotient, pitch perturbation quotient, amplitude perturbation quotient, harmonics-to-noise ratio, cepstral peak prominence, and long-term average spectrum were analyzed. All parameters improved after treatment, with a return to preparalytic values for most. During the first month, some deterioration was noted. This is the first study comparing a subject's own normal voice to his or her voice after vocal fold augmentation. We recommend overinjection of fat if vocal fold atrophy is expected.

KEY WORDS — acoustics, autologous fat, vocal fold paralysis.

INTRODUCTION

Variation in voice between individuals and within the same individual is the principal obstacle to the establishment of standardized objective voice analysis. Until now, all studies have based the objective voice results in the treatment of unilateral vocal fold paralysis (UVFP) on comparison to the voice before treatment, or on comparison with normal controls. We designed a study that allows comparison of a patient's own normal voice to his or her voice after the onset of UVFP and after treatment by autologous fat injection.

PATIENTS AND METHODS

A prospective study was carried out over a period of 7 months in an urban university hospital. All native French speakers scheduled for left pneumonectomy or lobectomy with mediastinal lymphadenectomy for bronchopulmonary carcinoma were examined and their voices were recorded before thoracic surgery. Patients with a history of laryngeal disease were excluded. Of the 25 patients included before operation, 2 male patients presented with iatrogenic left UVFP. These 2 patients are the subject of this report.

Both patients were clinically examined by fiberoptic laryngoscopy and stroboscopy before the onset of UVFP. In both patients, the laryngeal mobility was normal. There was no supraglottic activity on phonation and no evidence of laryngeal disease, in particular, leukoplakia or Reinke's edema, in these heavy smokers. The voice was recorded before the onset of UVFP and 1 week after its onset.

After the onset of UVFP, both patients presented with symptomatic aspiration of liquids with coughing. Aspiration was confirmed with videoendoscopic evaluation of swallowing using methylene blue. Because of the severe consequences of aspiration pneumonia in these patients, symptomatic treatment by vocal fold injection with autologous fat was performed 10 days after the thoracic surgery. The following technique was used. Fat was harvested from the abdomen through a 2-cm incision. Fascia and connective tissue were removed, and the fat was delicately minced with a steel blade.[1] The fat was then loaded into a 3-mL Brünings syringe with an 18-gauge needle. The injection was performed under general anesthesia, with apnea during injection. Two injections were performed: the first was placed 2 mm lateral to the vocal process, and the second was placed in front of the first at the midfold level. The depth of the injection was 5 mm, controlled by a mark on the needle. The volume injected (3 mL) was sufficient in both patients to produce a medially convex vocal fold, passing the midline.

A clinical evaluation using fiberoptic laryngoscopy with stroboscopy and voice recordings was performed 1 day (patient 1) to 3 days (patient 2) after fat injection and 1 month after injection. The aerodynamic eval-

---

From the Voice, Biomaterials and Head and Neck Oncology Research Laboratory, CNRS-UPRESA 7018, University Paris V, Georges Pompidou European Hospital (Hartl, Hans, Laccourreye, Brasnu), the Department of Thoracic Surgery, Georges Pompidou European Hospital (Riquet), and CNRS-UPRESA 7018, Institut de Linguistique et de Phonétique Générales et Appliquées, Université de la Sorbonne Nouvelle-Paris III (Vaissière), Paris, France.

CORRESPONDENCE — Daniel F. Brasnu, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France.

OBJECTIVE VOICE MEASUREMENTS

	Patient 1				*Patient 2*			
Parameter	*NL*	*UVFP*	*AF*	*1M*	*NL*	*UVFP*	*AF*	*1M*
PQuotient	0.35	0.44	0.29	0.58	0.23	0.59	0.44	0.35
PPQ	1.42	6.19	1.49	1.66	0.40	4.54	1.17	1.22
APQ	4.84	17.29	5.07	6.79	1.87	8.20	7.74	2.77
H/N	5.67	1.23	5.18	3.39	7.34	3.29	3.48	6.98
CPP	4.39	1.23	2.39	2.78	5.21	2.02	6.91	2.37
LTAS								
b0 – b1	15.38	10.90	15.85	13.86	15.03	9.72	16.82	12.06
b1 – b2	9.16	5.35	5.98	4.99	10.25	4.05	8.50	7.01
b2 – b3	6.98	4.86	5.86	3.67	7.82	4.23	9.63	5.39
b0 – b2	24.54	16.25	21.83	18.85	25.28	13.77	25.32	19.06
b0 – b3	31.52	21.12	27.69	22.53	33.10	18.00	34.95	24.45

PQuotient — phonation quotient in liters per second; PPQ — pitch perturbation quotient; APQ — amplitude perturbation quotient; H/N — harmonics-to-noise ratio; CPP — cepstral peak prominence; LTAS — long-term average spectrum in decibels (40 seconds of text; see text for definition of bands); NL — normal voice; UVFP — voice after onset of unilateral vocal fold paralysis; AF — voice immediately after augmentation with autologous fat; 1M — voice 1 month after augmentation with autologous fat.

uation was based on the phonation quotient, an indirect measure of phonatory air utilization, which is the maximum phonation time corrected for vital capacity. This correction was necessary because of lung resection. The maximum phonation time at a comfortable pitch and intensity (MPTc) for the vowel /a/ was measured manually from digital audiotape recordings. The vital capacity (VC) was measured with a Gould type II spirometer. The phonation quotient was equal to the VC divided by the MPTc.

For the acoustic analysis, samples of the voice were recorded in a quiet room. One track of a Sony DTC-60ES digital audiotape deck with a sampling rate of 48 kHz was used. The microphone was placed 15 cm in front of the patient and 5 cm above the level of the lips. The patient spoke at a comfortable level of intensity and pitch. We analyzed the subjectively most stable and voiced of 3 tokens of the vowel /a/ and 40 seconds of reading of a standardized text (French equivalent of the Rainbow Passage) of 100 words.

The samples were introduced by direct connection into a Sonograph model 4300B (Kay Elemetrics, Pine Brook, New Jersey) with resampling rates of 50 kHz for perturbation measures and 20 kHz for spectral measures. The pitch perturbation quotient (PPQ), amplitude perturbation quotient (APQ), and harmonics-to-noise ratio (H/N) were analyzed for a 1.75-second midvowel segment of the vowel /a/ by means of the Multidimensional Voice Program (MDVP version 1.34, Kay Elemetrics, 1993).

The cepstral peak prominence (CPP) was measured at the midpoint of 1 token of the vowel /a/. The cepstrum was calculated automatically from the Fourier transform spectrum (1,024-point hamming window, no preemphasis or smoothing) with a time range of 0.5 to 25.0 ms. The CPP corresponded to the relative energy level of the peak as compared to the regression line through the cepstral points.[2]

The spectra and spectrograms were calculated from a 1,000-ms midvowel segment of /a/ by means of the Computerized Speech Lab (CSL version 5.05, Kay Elemetrics, 1993) with a 1,024-point hamming window, without smoothing or preemphasis.

A long-term average spectrum (LTAS) was obtained for 40 seconds of text reading with the CSL software at a sampling rate of 50 kHz. No further editing of the text was performed after recording. The high-frequency intensity ratio, the ratio of the average spectral intensity level above 6 kHz to the average total intensity level, was calculated from data from the LTAS.[3] Low-, middle-, and high-frequency energy was measured by dividing the average spectrum of the text into 4 frequency bands.[4,5] Here, b0 was the average energy level for the points situated between 0 and 400 Hz; b1 was the average energy between 400 and 2,000 Hz; b2, between 2 and 5 kHz; and b3, between 5 and 10 kHz. The differences in intensity levels between these bands were calculated (see Table).

RESULTS

Fiberoptic laryngoscopy with stroboscopy was performed 7 days after thoracic surgery, after the onset of UVFP, revealing in both cases an immobile left vocal fold and arytenoid cartilage. In both cases the vocal process was in a paramedian position.[6] In patient 1, hooding by the cuneiform cartilage impeded the view of the posterior glottis. On phonation, a gap was visible between the membranous vocal folds. Supraglottic hyperactivity, with ipsilateral false vocal fold medialization and anteroposterior compression

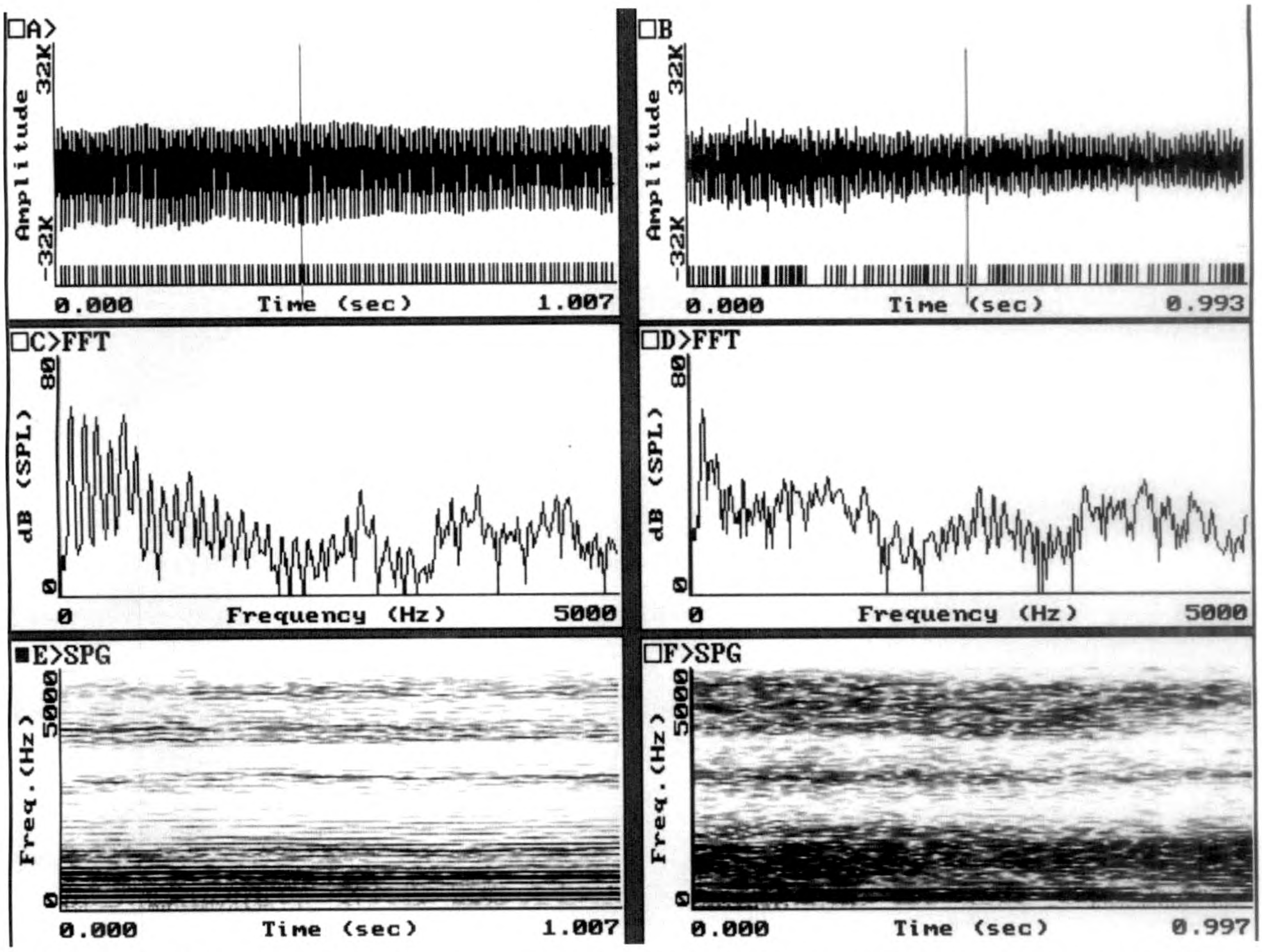


Fig 1. (Patient 2) Signals, spectra, and spectrograms of normal voice (left column) and those of voice after onset of unilateral vocal fold paralysis (right column). Windows A and B — signals; windows C and D — Fourier transform spectra; windows E and F — narrowband spectrograms.

of the supraglottic larynx, was observed (type III, according to Koufman's classification[7]). No mucosal wave was visible on the paralyzed vocal fold. In patient 2, the posterior glottis was visible, and no supraglottic hyperactivity was observed. On phonation, an elliptical gap was observed beween the membranous vocal folds, with asymmetric mucosal waves on stroboscopy. The posterior glottis was closed. On day 1 (patient 1) and day 3 (patient 2) after fat injection, the left vocal fold was convex medially, passing the midline. In patient 1, the supraglottic hyperactivity had completely disappeared, but the hooding of the posterior glottis persisted. In both patients, mucosal waves were visible on both vocal folds, but were asymmetric. One month after vocal fold augmentation, both patients had bowing of the left vocal fold. Patient 1 had supraglottic hyperactivity, with medialization of the ipsilateral false vocal fold (Koufman type II[7]). Posterior hooding persisted. In both patients, a glottal gap was present on phonation with present but asymmetric mucosal waves. In patient 2, the glottal gap involved only the membranous folds. Oral intake of liquids was resumed on the day after vocal fold injection, with no clinical aspiration. No aspiration was observed after 1 month.

Figures 1 and 2 show the visual representation of the signal, the spectrum, and the narrowband spectrogram for patient 2. The results for patient 1 were similar. A loss of signal periodicity was observed after the onset of UVFP (Fig 1), with a loss of harmonic energy, and an increase in the high-frequency energy seen on the spectrogram. A return of periodicity was observed after fat injection, persisting after 1 month (Fig 2). The harmonics were better-defined, and the high-frequency energy decreased, as seen on the spectra and the spectrograms after fat injection (Fig 2, lower left) and 1 month later (Fig 2, lower right).

For patient 1, the phonation quotient, PPQ, APQ, H/N, and b0 – b1 had improved to preparalytic values on day 1 after intrafold injection (see Table), while the CPP, b0 – b2, b0 – b3, and the high-frequency ratio (see Table and Fig 3) had improved without attaining preparalytic values. The voice was considered normal by the patient. At 1 month, all parameters had deteriorated, although only the phonation quo-

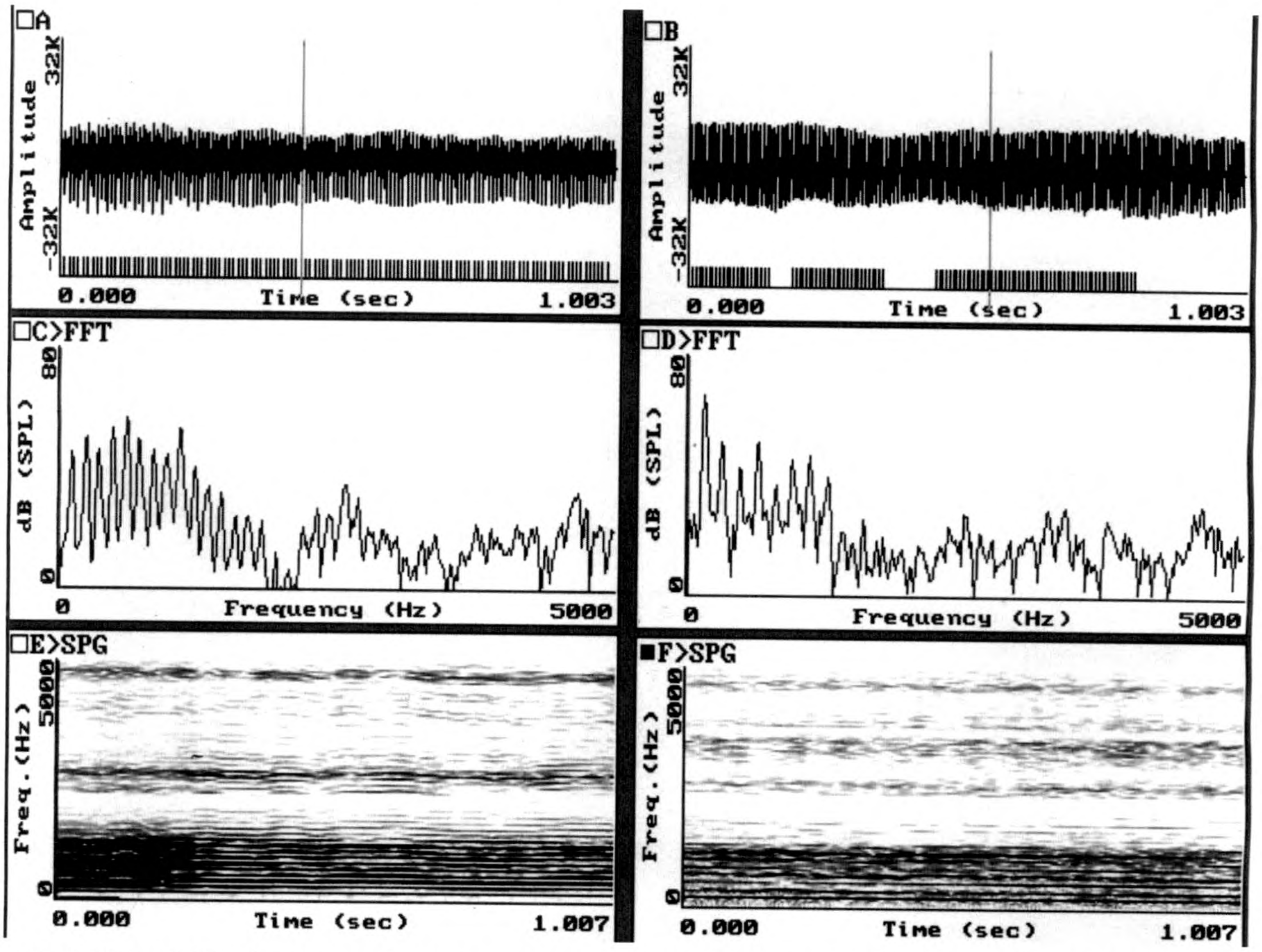


Fig 2. (Patient 2) Signals, spectra, and spectrograms of voice 3 days (left column) and 1 month (right column) after vocal fold augmentation with autologous fat. Windows A and B — signals; windows C and D — Fourier transform spectra; windows E and F — narrowband spectrograms.

tient and the high-frequency ratio had reverted to the levels measured before vocal fold injection. This evolution coincided with a subjective increase in phonatory effort and breathiness. For patient 2, the CPP, the spectral energy measures on LTAS (see Table), and the high-frequency ratio (Fig 3) improved to preparalytic values immediately after vocal fold augmentation. Like patient 1, patient 2 considered his voice to be normal. Unlike in patient 1, the phonation quotient, the perturbation measures (PPQ and APQ), and the H/N had improved 1 month after vocal fold augmentation. But, as in patient 1, the CPP, the spectral measures on LTAS, and the high-frequency ratio had deteriorated at 1 month. The evolution at 1 month coincided with a subjectively unstable voice quality, without an increase in phonatory effort or breathiness. Figure 4 gives a visual representation of the LTAS for patient 2, showing the relative decrease in middle- and high-frequency noise as compared to low-frequency harmonic levels after vocal fold augmentation; patient 1 had similar results.

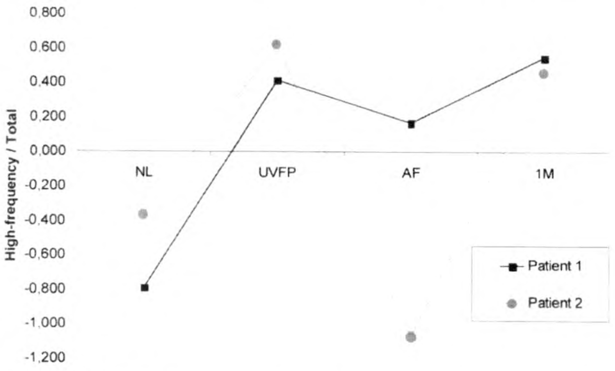
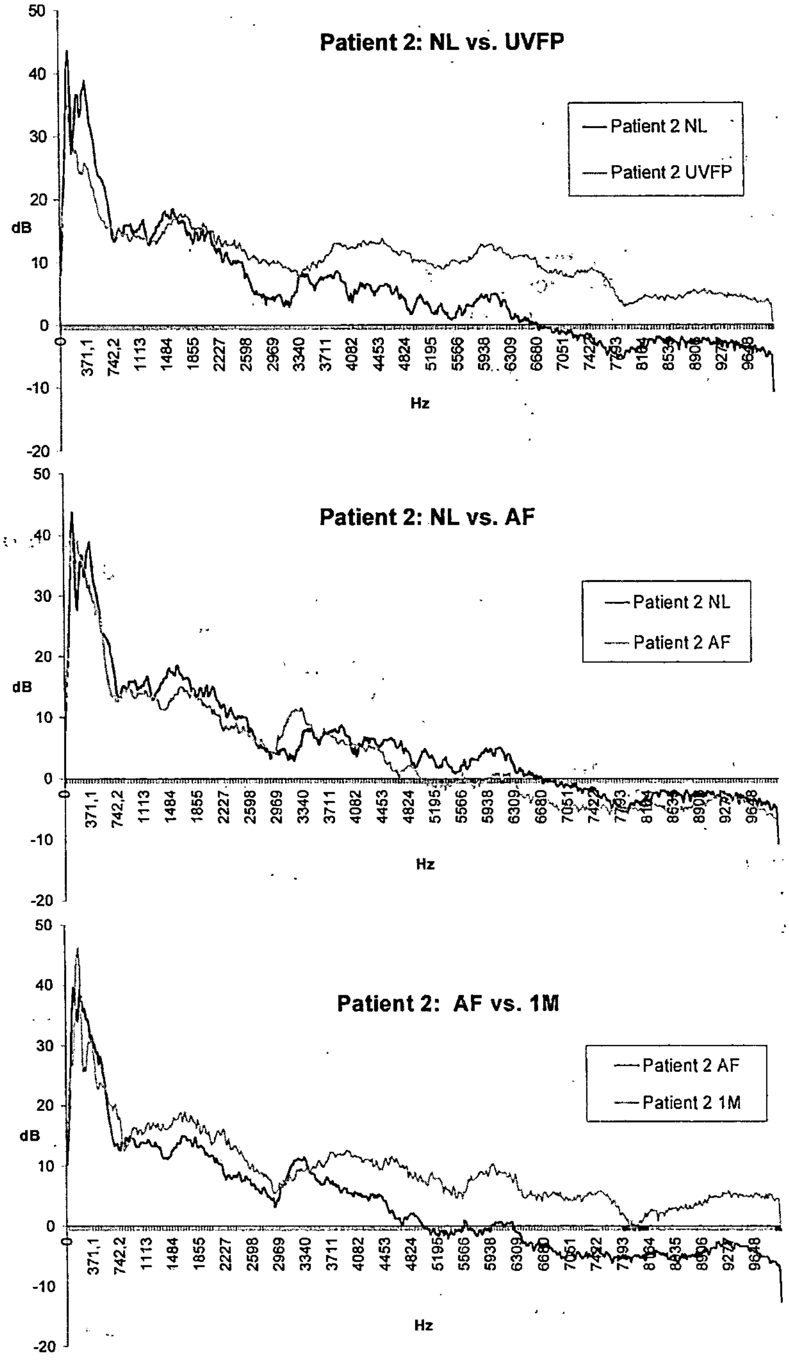


Fig 3. High-frequency intensity ratio. NL — normal voice; UVFP — unilateral vocal fold paralysis; AF — after vocal fold augmentation with autologous fat; 1M — 1 month after vocal fold augmentation with autologous fat.

DISCUSSION

Vocal fold augmentation using autologous fat was first introduced in 1991, by Mikaelian et al,[8] for the treatment of UVFP. Because of its effectiveness, availability, biocompatibility, low cost, and low rate of complications, it is now widely used.[1,9-11] The technique has been proven effective in treating aspi-



Patient 2: NL vs. UVFP

Patient 2: NL vs. AF

Patient 2: AF vs. 1M

Fig 4. (Patient 2) Long-term average spectra. At top is spectrum of normal voice (NL) versus that of unilateral vocal fold paralysis (UVFP, light tracing). At middle is spectrum of normal voice (NL) versus that of voice after vocal fold augmentation with autologous fat (AF, light tracing). At bottom is spectrum of voice after vocal fold augmentation with autologous fat (AF) versus that of voice 1 month after vocal fold augmentation (1M, light tracing).

ration caused by UVFP[1,11] and in improving the ease of phonation and vocal quality.[9,12,13]

To our knowledge, this is the first time a patient's own normal voice has been used as the reference for subsequent objective measures. This practice eliminates the interindividual variability of voice and facilitates the evaluation of treatment outcome, eliminating the problem of the overlap in voice parameters between normal and dysphonic populations.[8,14]

We were able to validate acoustic and aerodynamic findings of previous reports concerning the results of treatment of UVFP. All of the parameters measured in this study had previously been correlated to perceived breathiness in normal subjects and in dysphonic patients. The perturbation measurements PPQ and APQ, traditionally associated with roughness, have also been shown to correlate to breathiness.[5,14] In other studies, the H/N and the high-frequency ratio corresponded to perceived breathiness,[3,5] and the CPP correlated inversely to breathiness.[2] In breathy voices, others have shown a relative increase in the middle- and high-frequency energy bands of the LTAS.[15,16] Finally, breathiness has been correlated to an increase in phonatory airflow and a decrease in maximum phonation time.[3,17]

After treatment of UVFP, various reports have found significant improvement in objective parameters as compared to paralytic values. The acoustic results of treatment include a decrease in pitch and amplitude perturbation[12,13,18,19] and an increase in CPP.[19] Treatment caused a decrease in noise and an increase in harmonic levels,[13,20] and a visible improvement of sound spectrograms.[15] Aerodynamic analysis showed a decrease in phonation quotient and an increase in maximum phonation time.[9]

Today, essentially 3 groups of surgical techniques are used for the symptomatic treatment of UVFP: 1) vocal fold augmentation by injection of various substances (Teflon, silicone, Gelfoam, GAX collagen, autologous fat), 2) laryngeal framework surgery (medialization thyroplasty, arytenoid adduction), and 3) laryngeal reinnervation (end-to-end anastomosis, muscular patch, laryngeal pacing). Concerning the injection techniques using Teflon and collagen, Rontal and Rontal have stated that "a perfect voice that is completely restored to preparalysis condition is rare and not to be expected."[21(p1148)] In our study, however, we observed a complete return to preparalytic values for almost all of the parameters tested. The viscosity of the injected material probably contributes to the vibratory results. In an in vitro study, fat seemed to have the most "physiologic" viscosity of all substances available at present,[22] possibly explaining the good objective results.

After injection, the noise traditionally associated with a turbulent glottic air leak decreased as compared to the harmonic energy as measured by b0 – b2 and b0 – b3 (see Table) and by the high-frequency intensity ratio (Fig 3). One month later, the spectral improvement had started to deteriorate. This coincided in both patients with the onset of left vocal fold bowing, whereas the vocal fold had been convex immediately after injection. Shindo et al[1] also found that voice quality gradually deteriorated during the first, second, and third months, then stabilized between the third month and 6 to 12 months after fat injection. Resolution of the postinjection edema[21] and the onset of vocal fold atrophy[1] are probably the main factors in this voice deterioration. Vocal fold atrophy occurs within 2 to 3 weeks after nerve section[23] and was probably responsible for the vocal fold bowing seen at 1 month. This evolution could not have been foreseen in our 2 cases, because the exact mechanism of UVFP during thoracic surgery was unknown. (The recurrent laryngeal nerve was not identified during surgery.) If the mechanism had been simple neurapraxia, we could have expected a preservation of vocal fold muscle tone. The onset of atrophy meant that the mechanism was axonotmesis or neurotmesis, more severe lesions to the recurrent laryngeal nerve.[23] An initial overinjection of fat could have, in theory, limited the vocal deterioration. Resorption of fat is believed to take place after approximately 5 months,[10,24] although some reports have indicated a long-term improvement of more than 1 year.[1,8,9] Thus, resorption of fat is not likely to have caused the voice deterioration seen at 1 month in our 2 patients.

The inability to close the posterior glottis with the injection techniques is a factor contibuting to persistent breathiness.[25] In patient 1, hooding precluded visual evaluation of the posterior glottis, but the supraglottic hyperfunction could be interpreted as an indirect sign of aerodynamic glottic incompetence.[26]

In patient 2, the deterioration of the spectral components observed at 1 month coincided with a slight deterioration in subjective voice quality. However, by 1 month, the perturbational measures and the H/N had improved as compared to the immediate postinjection measurements. Laryngeal aerodynamic adjustments could be responsible for this delay in improvement. We have no satisfying explanation for the simultaneous deterioration of spectral measurements and improvement of perturbational measurements. It is also possible that the automatic detection of pitch decreases the sensitivity of measures such as the PPQ, APQ, and H/N for dysphonic voices.[5,27] Whatever the explanation, this variability in evolution underlines the variability of voice that exists, even within the same subject. Because of the lack of specificity and sensitivity of any one individual measure, most authors advocate using several complementary parameters to objectively measure voice quality,[1,12,17,20] as we have.

The short follow-up of these 2 patients (1 month) was due to the morbidity of the pulmonary carcinoma. Both were lost to voice follow-up because of deterioration of their general health and the morbidity of their adjuvant treatment regimens (radiotherapy and chemotherapy). Given the limited life expectancy of patients with pulmonary carcinoma, the duration of the effects of fat injection may be sufficient.[18,28] The fat does not interfere with an eventual spontaneous recovery of laryngeal function (with or without synkinesis).[29] Also, this technique does not preclude a more permanent treatment (thyroplasty) if it becomes necessary.[30]

CONCLUSION

This comparison of normal voice, voice after onset of UVFP, and voice after vocal fold augmentation with autologous fat within the same patients confirms the usefulness of fat injection for improving the voice in patients with UVFP. Objective and subjective voice parameters improved, with an almost complete return to preparalytic values. Voice deterioration was observed at 1 month and was probably due to vocal fold atrophy. We recommend overinjection of fat when atrophy is expected. Because of the extreme variability of the voice, even within the same individual, we recommend using several types of parameters (aerodynamic, spectral, and perturbational) to objectively evaluate the vocal results in the treatment of UVFP. Obtaining voice samples before surgery in patients at risk for UVFP is helpful in evaluating the treatment outcome.

REFERENCES

1. Shindo ML, Zaretsky LS, Rice DH. Autologous fat injection for unilateral vocal fold paralysis. Ann Otol Rhinol Laryngol 1996;105:602-6.

2. Hillenbrand J, Cleveland RA, Erickson RL. Acoustic correlates of breathy vocal quality. J Speech Hear Res 1994;37:769-78.

3. Shoji K, Regenbogen E, Yu JD, Blaugrund SM. High-frequency power ratio of breathy voice. Laryngoscope 1992;102:267-71.

4. Hammarberg B, Fritzell B, Schiratzki H. Teflon injection in 16 patients with paralytic dysphonia: perceptual and acoustic evaluations. J Speech Hear Disord 1984;49:72-82.

5. deKrom G. Some spectral correlates of pathological breathy and rough voice quality for different types of vowel fragments. J Speech Hear Res 1995;38:794-811.

6. Woodson GE. Configuration of the glottis in laryngeal paralysis. I: Clinical study. Laryngoscope 1993;103:1227-33.

7. Koufman JA. Approach to the patient with a voice disorder. Otolaryngol Clin North Am 1991;24:989-98.

8. Mikaelian DO, Lowry LD, Sataloff RT. Lipoinjection for unilateral vocal cord paralysis. Laryngoscope 1991;101:465-8.

9. Brandenburg JH, Kirkham W, Koschkee D. Vocal cord augmentation with autogenous fat. Laryngoscope 1992;102:495-500.

10. Bauer CA, Valentino J, Hoffman HT. Long-term result of vocal cord augmentation with autogenous fat. Ann Otol Rhinol Laryngol 1995;104:871-4.

11. Laccourreye O, Paczona R, Ageel M, Hans S, Brasnu D, Crevier-Buchman L. Intracordal autologous fat injection for aspiration after recurrent nerve paralysis. Eur Arch Otorhinolaryngol 1999;256:458-61.

12. Adams SG, Irish JC, Durkin LC, Wong DLH, Brown DH. Evaluation of vocal function in unilateral vocal fold paralysis following thyroplastic surgery. J Otolaryngol 1996;25:165-70.

13. Ford CN, Bless DM, Loftus JM. Role of injectable collagen in the treatment of glottic insufficiency: a study of 119 patients. Ann Otol Rhinol Laryngol 1992;101:237-47.

14. Hirano M, Hibi S, Yoshida T, Hirade Y, Kasuya H, Kikuchi Y. Acoustic analysis of pathological voice. Acta Otolaryngol (Stockh) 1988;105:432-8.

15. Kim KM, Kakita Y, Hirano M. Sound spectrographic analysis of the voice of patients with recurrent laryngeal nerve paralysis. Folia Phoniatr 1982;34:124-33.

16. Mendoza E, Valencia N, Muñoz J, Trujillo H. Differences in voice quality between men and women: use of the long-term average spectrum (LTAS). J Voice 1996;10:59-66.

17. Hirano M, Mori K, Tanaka S, Fujita M. Vocal function in patients with unilateral vocal fold paralysis before and after silicone injection. Acta Otolaryngol (Stockh) 1995;115:553-9.

18. Laccourreye O, Hans S, Menard M, Haquart N, Brasnu D, Crevier-Buchman L. Résultats de l'injection intracordale de graisse autologue dans les paralysies laryngées postchirurgicales. Chirurgie 1999;124:283-7.

19. Olson DEL, Goding GS, Michael DD. Acoustic and perceptual evaluation of laryngeal reinnervation by ansa cervicalis transfer. Laryngoscope 1998;108:1767-72.

20. Hirano M, Tanaka S, Tanaka Y, Hibi S. Transcutaneous intrafold injection for unilateral vocal fold paralysis: functional results. Ann Otol Rhinol Laryngol 1990;99:598-604.

21. Rontal E, Rontal M. Vocal cord injection techniques. Otolaryngol Clin North Am 1991;24:1141-9.

22. Chan RW, Titze IR. Viscosities of implantable biomaterials in vocal fold augmentation surgery. Laryngoscope 1998;108:725-31.

23. Kline DG. Basic considerations in nerve physiology. In: Kline DG, Hudson AR, New E, eds. Nerve injuries: operative results for major nerve injuries, entrapments and tumors. 1st ed. Philadelphia, Pa: WB Saunders, 1995:10-25.

24. Saccogna PW, Werning JW, Setrakian S, Strauss M. Lipoinjection in the paralyzed feline vocal fold: study of graft survival. Otolaryngol Head Neck Surg 1997;117:465-70.

25. Dedo HH. Injection and removal of Teflon for unilateral vocal cord paralysis. Ann Otol Rhinol Laryngol 1992;101:81-6.

26. Von Doersten PG, Izdebski K, Ross JC, Cruz RM. Ventricular dysphonia: a profile of 40 cases. Laryngoscope 1992;102:1296-301.

27. Inagi K, Khidr AA, Ford CN, Bless DM, Heisey DM. Correlation between vocal functions and glottal measurements in patients with unilateral vocal fold paralysis. Laryngoscope 1997;107:782-91.

28. Gardner GM, Shaari CM, Parnes SM. Long-term morbidity and mortality in patients undergoing surgery for unilateral vocal cord paralysis. Laryngoscope 1992;102:501-8.

29. Laccourreye O, Crevier-Buchman L, Le Pimpec-Barthes F, Garcia D, Brasnu D, Riquet M. Recovery of function after intracordal autologous fat injection for unilateral recurrent nerve paralysis. J Laryngol Otol 1998;112:1082-4.

30. Hoffman HT, McCulloch TM. Anatomic considerations in the surgical treatment of unilateral laryngeal paralysis. Head Neck 1996;18:174-87.

Ann Otol Rhinol Laryngol 110:2001

ENHANCEMENT OF SUBMICROSCOPIC DAMAGE OF THE NASAL EPITHELIUM BY TOPICAL ALLERGEN CHALLENGE IN PATIENTS WITH PERENNIAL NASAL ALLERGY

HIROSHI NAGATA, MD, PHD
HIDEAKI MOTOSUGI, MD, PHD
AKIKO SANAI, MD, PHD
HOMARE SUZUKI, MD, PHD
KAZUTO OHNO, MD, PHD
TSUTOMU NUMATA, MD, PHD
AKIYOSHI KONNO, MD, PHD
CHIBA, JAPAN

The purposes of this study were to clarify whether damage of the nasal epithelium exists in patients with nasal allergy, and how the morphology of the epithelium changes after topical allergen challenge. Electron microscopy revealed 2 characteristic features in the nasal epithelium of patients with perennial nasal allergy — an increase in the number of epithelial cells with cytoplasmic vacuoles, and markedly widened intercellular spaces — although these changes were unclear under light microscopy. The density of vacuolated cells significantly increased 24 hours after allergen challenge. Further, the number of eosinophils that were associated with vacuolated cells was significantly higher in patients with nasal allergy than in controls. These morphological changes, thus, were considered to be types of damage to the nasal epithelium associated with nasal allergy. Such changes may be among the causes of nasal hyperreactivity, which is an important feature of nasal allergy.

KEY WORDS — damage, electron microscopy, nasal allergy, nasal epithelium, quantitative study.

INTRODUCTION

Airway hyperreactivity is a common and important feature of allergic inflammation of the airway. Allergen-induced hyperreactivity in the nose was first described by Connell,[1] who observed an increase in the reactivity of the nasal mucosa in patients with pollinosis following repeated exposures to pollen — an increase that was reversible in days or weeks. Likewise, in a natural setting, patients with pollinosis have more pronounced symptoms in response to the same amount of histamine in the season of pollinosis as compared with the off-season.[2] Thus, repeated provocation of nasal mucosa with an allergen causes hyperreactivity of the mucosa to histamine as well as allergens; the underlying mechanisms are unknown.

In bronchial asthma, it is considered that one of major causes of hyperreactivity is damage of the bronchial epithelium, which is believed to permit exposure of the C fiber terminals and elevation of mucosal permeability. Several investigators have shown that there is destruction of the bronchial epithelium, such as epithelial cell shedding and widening of intercellular spaces,[3-8] and that the degree of epithelial damage correlates with the degree of airway reactivity.[7,8] Eosinophils are considered responsible for this destruction of the bronchial epithelium. Several studies have reported that the degree of eosinophil infiltration correlates with the extent of epithelial damage.[8-10] Moreover, Filley et al[4] have shown that there was a deposition of major basic protein (MBP), a cationic protein of eosinophils that is considered toxic for tissue, on the damaged bronchial epithelium of a patient who had died of asthma. In contrast, the presence of damage of the nasal epithelium in nasal allergy is still controversial, although infiltration of eosinophils is a characteristic feature of nasal allergy as well. In an electron microscopic study, Toppozada and Talaat[11] found that the nasal epithelium associated with chronic perennial nasal allergy showed widened intercellular spaces — a change similar to that observed in the bronchial epithelium in asthma.[8] On the other hand, by light microscopy, Kawabori et al[12] found that epithelial shedding was rarely observed in the nasal epithelium in perennial allergic patients, and was not related to the tissue density of eosinophils or the degree of activation of eosinophils. Thus, for understanding how hyperreactivity of the nasal mucosa is elicited in nasal allergy, it still needs to be clarified whether damage of the nasal mucosa is caused by allergic inflammation.

Thus, the purposes of this study were to clarify whether damage of the nasal epithelium exists in patients with nasal allergy, and to clarify how the morphology of the epithelium changes after topical allergen challenge. Further, we aimed to determine wheth-

---

From the Department of Otorhinolaryngology, Chiba University School of Medicine, Chiba, Japan.

CORRESPONDENCE — Hiroshi Nagata, MD, PhD, Dept of Otorhinolaryngology, Chiba University School of Medicine, 1-8-1 Inohana, Chuoh-ku, Chiba 260-8670, Japan.

er epithelial damage is associated with eosinophil infiltration.

MATERIALS AND METHODS

Subjects. We enrolled 17 patients who were admitted to Chiba University Hospital to undergo conchotomy for intractable nasal obstruction. Four control patients (ages 15 to 42 years; average, 30.0 years) suffered from chronic hypertrophic rhinitis but not nasal allergy. The other 13 patients (ages 14 to 35 years; average, 19.5 years) suffered from perennial nasal allergy and showed positive skin tests to house dust and mites. These patients were divided into 3 groups randomly. They were intranasally challenged with control disks or allergen disks, which were filter papers 3 mm in diameter and containing 250 μg allergen (3.7 μg protein nitrogen) extracted from house dust (Torii Pharmaceuticals, Tokyo, Japan). The first group (n = 4) was challenged with control disks 24 hours before surgery, the second group (n = 4) was challenged with allergen disks 8 hours before surgery, and the third group (n = 5) was challenged with allergen disks 24 hours before surgery. The allergen challenge was done by putting 3 disks onto the inferior turbinates. Surgery was performed under general anesthesia with no topical application of drugs. Informed consent to study participation was obtained from all the patients before the study.

Electron Microscopy. The nasal mucosae obtained by surgery were cut into small pieces immediately after excision and fixed with 2.5% glutaraldehyde and 1.0% paraformaldehyde in 0.1-mol/L phosphate buffer overnight. After brief washing in the same buffer, the tissues were postfixed with 40-mmol/L osmium tetroxide in 0.1-mol/L cacodylate buffer for 1 hour at 4°C. They were then dehydrated through a series of ethanols and embedded in epoxy resin. Sections for light microscopy were cut on a Sorvall MT-1 ultramicrotome and stained with 0.1% toluidine blue. To exclude artifacts caused by the surgical procedures, we trimmed portions denuded of epithelium off the epoxy blocks. Ultrathin sections were cut on an Ultracut E ultramicrotome (Reichert-Jung, Vienna, Austria) and mounted on a support of a Formvar film without grids (Fig 1A). After staining with uranyl acetate followed by lead citrate, the whole section was photographed at a magnification of 500× with a JEOL (Tokyo) 1200EX electron microscope. For the quantitative studies, a picture was printed to a 30.5 × 25.4-cm bromide paper (Fuji Film, Tokyo) at a final magnification of 1,500×, so that 1 picture covered an area of approximately 200 μm in width and 165 μm in height. One electron microscopic section included 98 to 183 apical epithelial cells, which included ciliated cells, cylindrical cells, and goblet cells, and 2 or 3 pictures were enough for observation of a whole section. The morphology of apical epithelial cells was examined carefully by means of a magnifier. Then, the numbers of apical cells (total) and of degraded cells that showed vacuolation in the cytoplasm were counted, and the ratio of the latter to the former was obtained. Further, the number of widened intercellular spaces in 100 apical cells was calculated. In addition, the number of eosinophils that had infiltrated into the apical epithelial layer was counted, and the ratio of eosinophils that made contact with a vacuolated cell(s) was obtained. These data from the 4 patient groups were compared with each other statistically with the Mann-Whitney U-test. Further, the correlation between the ratio of vacuolated cells to total apical cells and the number of widened intercellular spaces in 100 apical cells was tested with Pearson's correlation coefficient.

Immunohistochemistry. For immunohistochemical analysis of the MBP of eosinophils, the excised nasal mucosae were embedded in Tissue-Tek OCT Compound (Sakura Finetek, Torrance, Calif) and snap frozen in liquid nitrogen. Sections 8 μm thick were cut on a cryostat and mounted on slides coated with poly-L-lysine. The sections were then fixed in 50% ethanol for 15 minutes at room temperature and incubated with 1% hydrogen peroxide in phosphate-buffered saline solution (PBS) for 15 minutes at room temperature. After thorough washing with PBS, the sections were preincubated with a blocking solution containing 1% skim milk, 0.3% Triton X-100, and 0.1% sodium azide in PBS for 60 minutes at room temperature, and then incubated with a mouse monoclonal antibody for human MBP (BMK-13, Nichirei, Tokyo; 1:20 dilution in the blocking solution) at 4°C overnight. Positive immunoreactivity was detected by 2 different methods: fluorescent microscopy and diaminobenzidine (DAB) histochemical analysis for horseradish peroxidase (HRP). After washing in PBS, the sections were incubated with rabbit anti-mouse IgG conjugated with Texas red (Organon Teknika, Durham, NC; 1:100 dilution in the blocking solution) and examined on a fluorescence microscope. The other sections were incubated with biotinylated rabbit anti-mouse IgG followed by streptavidin-HRP complex by means of a Histofine kit (Nichirei). The sections were then washed in Tris buffer (pH 7.6), reacted with 0.02% DAB solution containing 0.25% ammonium nickel sulfate hexahydrate and 0.0006% hydrogen peroxide for 8 to 14 minutes at room temperature, and examined under a light microscope.

RESULTS

Electron Microscopy. To avoid mechanical artifacts on the morphology of the nasal epithelium, we per-

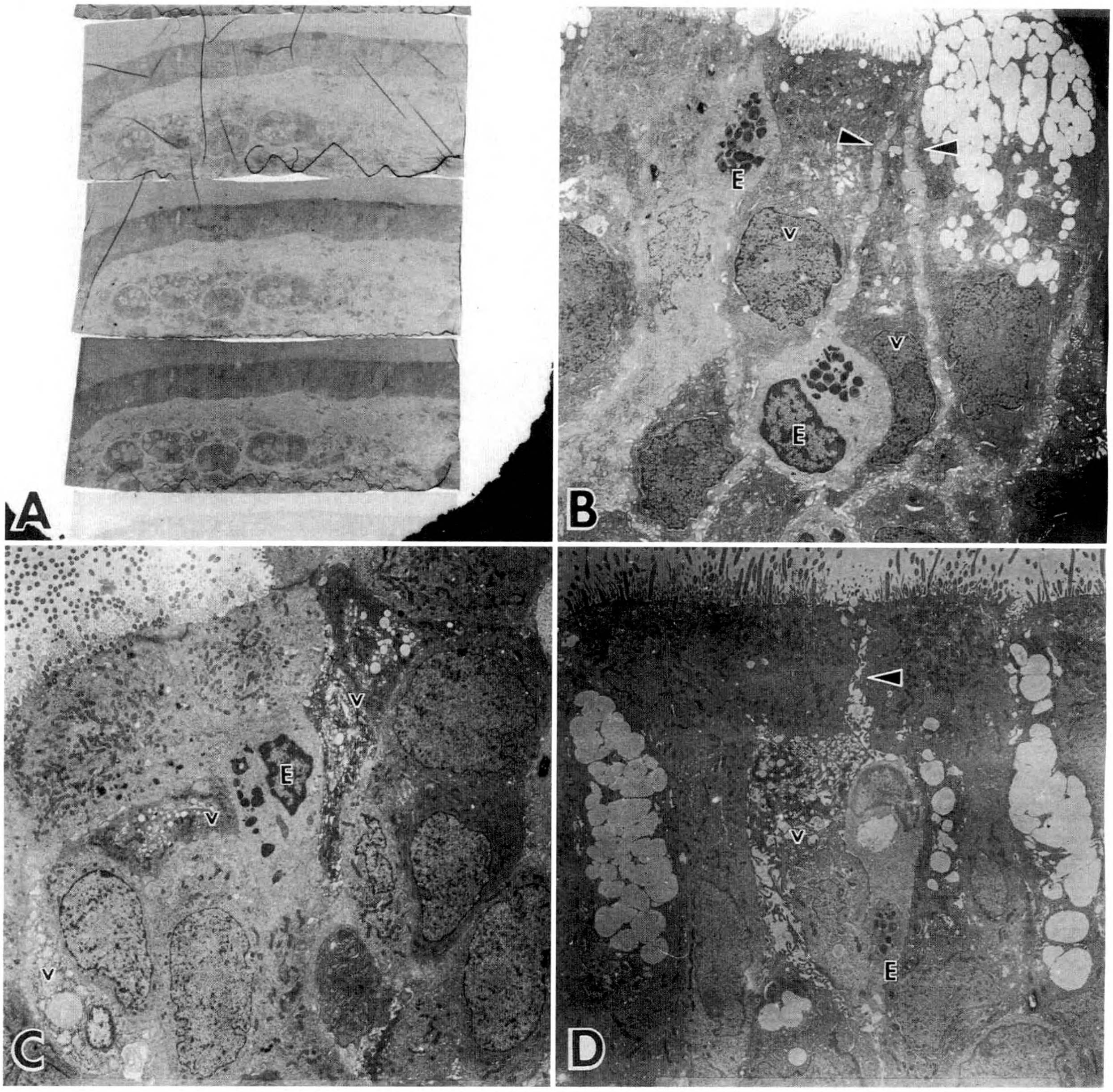


Fig 1. Electron micrographs of nasal mucosae from patients with nasal allergy. A) Low-power photograph of serial ultrathin sections mounted on support of Formvar film (original ×120). Because there is no grid, whole section can be examined with no difficulty. B) Nasal mucosa challenged with control disks (original ×2,400). There are 2 eosinophils (E) and 2 epithelial cells, which have small vacuoles in their cytoplasm (v). Widened intercellular spaces (arrowheads) are noted to be filled with electron-dense material. C,D) Nasal mucosae of patients challenged with allergen 24 hours before surgery (original ×2,400). Epithelial cells with marked vacuolation (v) and eosinophils (E) are observed. Widened intercellular space (arrowhead) is noted.

formed electron microscopic observation on a portion that showed no obvious denudation of the epithelium under light microscopy. Electron microscopy revealed 2 characteristic changes in the nasal epithelium of patients who had nasal allergy: an increase in the number of epithelial cells with cytoplasmic vacuoles, and markedly widened intercellular spaces (Fig 1B-D). The vacuolated cells were usually found alone among the normal-appearing epithelial cells, and were frequently accompanied by eosinophils. Moreover, these cells were occasionally associated with considerably widened intercellular spaces forming large gaps between epithelial cells. The gaps were found to measure up to 1.5 μm, whereas the normal apical intercellular spaces were approximately 25 nm in width. However, tight junctions usually remained closed (Fig 1B,D).

One electron microscopic section included 98 to 183 apical epithelial cells, and the results of the quantitative study are summarized in the Table and Fig 2. The ratio of vacuolated cells to the total number of

QUANTITATIVE ELECTRON MICROSCOPIC ANALYSIS OF NASAL EPITHELIA IN CONTROLS AND PATIENTS WITH NASAL ALLERGY

Patient	*Ratio* of Vacuolated Cells to Apical Epithelial Cells*	*No. of Widened Intracellular Spaces in 100 Apical Epithelial Cells*	*Ratio* of Eosinophils Associated With Vacuolated Cells to Total Eosinophils in Apical Epithelial Cell Layer*
Control patients (n = 4)			
1	1.0 (1/99)	0	NE
2	1.9 (2/103)	0	0 (0/1)
3	0 (0/98)	0	NE
4	0 (0/127)	0	0 (0/1)
Patients with nasal allergy			
Challenged with control disks (n = 4)			
5	11.1 (12/108)	7.8	41.6 (10/24)
6	11.4 (12/105)	1.9	66.7 (2/3)
7	9.2 (10/109)	4.6	NE
8	9.2 (10/109)	6.4	NE
Challenged with allergen disks 8 h before surgery (n = 4)			
9	13.6 (16/118)	16.1	100 (5/5)
10	10.3 (12/116)	6.0	NE
11	7.5 (9/120)	0	NE
12	20.3 (22/108)	9.2	100 (6/6)
Challenged with allergen disks 24 h before surgery (n = 5)			
13	18.0 (32/183)	3.3	100 (3/3)
14	38.0 (12/173)	20.2	77.8 (7/9)
15	10.9 (12/119)	7.6	NE
16	22.6 (12/150)	10.7	NE
17	24.0 (12/121)	20.6	66.7 (2/3)

NE — no eosinophils observed in epithelium.

*Expressed as percentage, with ratio in parentheses.

apical cells was statistically significantly greater in patients with nasal allergy than in the controls (see Table and Fig 2A). Further, the density of the vacuolated cells was significantly increased for the patients who were challenged with allergen disks 24 hours before surgery, compared with the density for patients challenged with control disks (see Table and Fig 2A). Likewise, the number of widened intercellular spaces in 100 apical epithelial cells was significantly greater in the patients with nasal allergy than in the controls (see Table and Fig 2B). Moreover, the ratio of vacuolated cells to the total number of apical cells correlated to the number of widened intercellular spaces with a correlation coefficient of .838

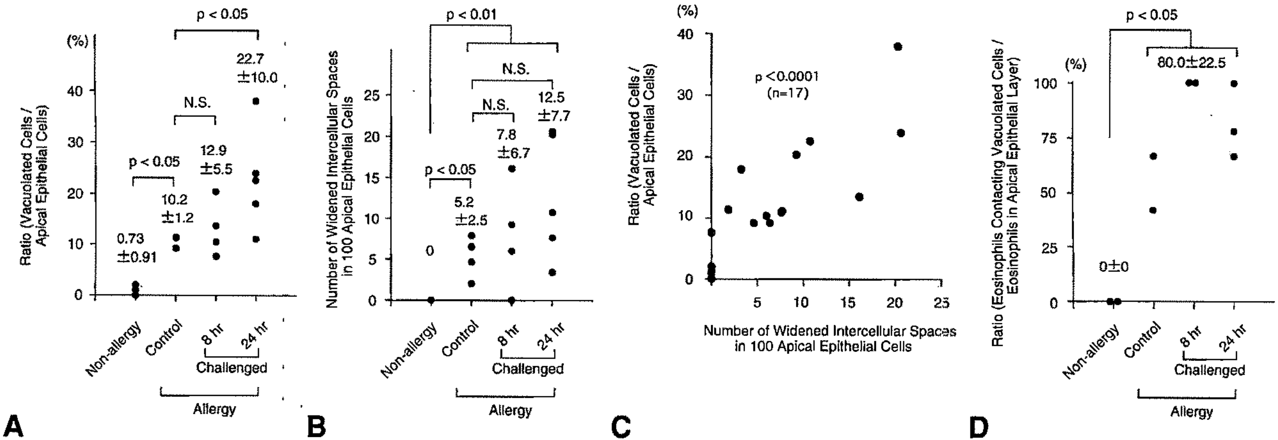


Fig 2. Differences in morphology of nasal mucosa in nonallergic and allergic patients, and effects of allergen challenge. Values are mean ± SD. A) Ratio of vacuolated cells to apical epithelial cells. B) Number of widened intercellular spaces in 100 apical epithelial cells. C) Correlation between ratio of vacuolated cells in apical epithelial cells and number of widened intercellular spaces in 100 apical epithelial cells. D) Percentage of eosinophils that make contact with vacuolated cell(s). Intraepithelial eosinophils could be found in 2, 2, 2, and 3 subjects in 4 groups.

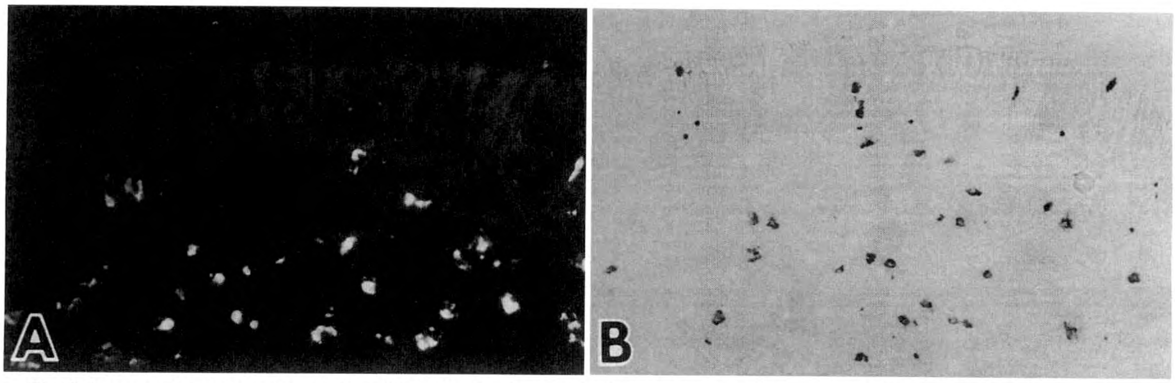


Fig 3. Immunohistology for eosinophil major basic protein in allergic nasal mucosa challenged with allergen disks 24 hours before surgery. A) Fluorescent microscopy (original ×650). B) Diaminobenzidine immunohistochemistry (original ×450).

($p < .0001$, Fig 2C). However, no statistical difference in the number of widened intercellular spaces in 100 apical cells was observed among the 3 patient groups with nasal allergy (see Table and Fig 2B). The number of eosinophils was counted in 2, 2, 2, and 3 subjects in the 4 groups, and the proportion of eosinophils that were associated with vacuolated cells was found to be significantly higher in the patients with nasal allergy than in the controls (see Table and Fig 2D).

Immunohistochemistry. We used 2 sensitive immunohistochemical techniques for detecting MBP in the nasal mucosa: fluorescent microscopy and light microscopy for DAB immunohistochemistry (Fig 3). Both techniques showed positive staining for MBP, although this seemed to be mostly confined to eosinophils. Neither of the techniques showed large deposits of MBP on the subepithelial interstitium or the surface of the nasal mucosa. However, small, spotlike deposits were observed in the submucosa, as well as in the epithelial layer.

DISCUSSION

The present study reveals that there is damage of the nasal epithelium at an electron microscopic level in patients with nasal allergy, although this damage is not clear under light microscopy. The characteristics of the morphological changes in the nasal epithelium in patients with nasal allergy were an increase in the number of vacuolated cells and widened intercellular spaces in the apical cell layer. Further, the density of vacuolated cells was significantly increased 24 hours after topical allergen challenge — a finding suggesting that the generation of vacuolated cells is due to a local allergic reaction and/or subsequent allergic inflammation. Moreover, the widening of the intercellular spaces is probably a secondary change following the generation of the vacuolated cells, because these spaces were usually found adjacent to those vacuolated cells, and the ratio of vacuolated cells to the total number of apical cells correlated to the number of widened intercellular spaces. The presence of damage of the nasal epithelium in nasal allergy has been controversial. Toppozada and Talaat[11] have reported the electron microscopic observation that the nasal epithelium associated with chronic perennial nasal allergy showed widened intercellular spaces — a change similar to that observed in this study. On the other hand, Kawabori et al[12] reported that epithelial shedding was rarely observed in the nasal epithelium in perennial allergic patients. Therefore, these findings, together with our electron and light microscopic findings, strongly suggest that the damage to the nasal epithelium in nasal allergy is usually of the sort that is recognized only at the electron microscopic level, and this damage may occasionally lead to a large desquamation of epithelium.

The present study showed that the proportion of eosinophils that were associated with vacuolated cells was statistically significantly higher in patients with nasal allergy than in the controls — a finding suggesting that the generation of epithelial damage in nasal allergy is related to infiltrating eosinophils. Likewise, in bronchial asthma, several studies have reported that the degree of eosinophil infiltration correlates with the extent of epithelial damage.[8-10] Moreover, Filley et al[4] have demonstrated a remarkable deposition of MBP on the damaged bronchial epithelium of a patient who died of asthma. Thus, eosinophils have been considered to play a role in destroying the bronchial epithelium, particularly in asthma, with its cationic proteins. The present study failed to show remarkable deposits of MBP. Instead, we found only small ones on the epithelium and on the interstitium. In nasal allergy, Kawabori et al[12] have reported that epithelial shedding was observed only in some areas and was not related to the tissue density of eosinophils or to the degree of activation of eosinophils. Thus, further studies should be performed to establish the role of eosinophils in damaging the nasal epithelium in nasal allergy. In addressing this problem, we have observed that an allergen challenge changes the morphology and expression of the cell adhesion molecule E-cadherin of the nasal epithelium in a guinea pig model of nasal allergy.[13] In that study, we observed that a topical allergen challenge induced marked infiltration of eosinophils, widening of the intercellular spaces, a loss of E-cadherin immunoreactivity around the infiltrating eosinophils, and small deposits of cationic proteins. Therefore, we believe that eosinophils play a role in damaging the nasal epithelium in guinea pigs, as well as in human nasal allergy, by the direct effect of intraepithelial migration and/or by the effect of cationic proteins. However, the degree of their toxic effects in nasal allergy is probably weaker than that reported for bronchial asthma.

In bronchial asthma, several investigators have shown that the degree of epithelial damage correlates with the degree of airway reactivity[7,8] and that one of the major causes of hyperreactivity is damage of the bronchial epithelium. Likewise, widening of intercellular spaces and an increase in the number of degraded epithelial cells may elevate nasal reactivity. Several mechanisms can be proposed to explain the relationship between epithelial damage and nasal hyperreactivity. First, damage of the nasal epithelium probably leads to a loss of barrier function, allowing a leakage of water and solutes, including various chemical mediators, into the nasal cavity and the penetration of inhaled material to the nasal interstitium. Indeed, nasal mucosal permeability to macromolecules such as albumin[14] and HRP[15] was reported to be increased in patients with allergic rhinitis as compared with normal subjects. Second, an increase in the number of vacuolated cells may result in a loss of the active defense mechanism of the nasal epithelium; for example, epithelial neutral endopeptidase and angiotensin-converting enzyme are considered to be important for degrading proinflammatory neuropeptides.[16,17] Third, epithelial damage may expose intraepithelial sensory nerves, permitting these nerves to be easily stimulated by inflammatory mediators. Our previous study, using a guinea pig model of nasal allergy, showed that capsaicin-sensitive sensory nerves constitute an afferent pathway of the sneezing reflex in nasal allergy.[18] To establish a relationship between damage and reactivity of the nasal mucosa, further studies, evaluating the effects of allergen challenge on nasal reactivity, are required.

In conclusion, the present study showed damage of the nasal epithelium in nasal allergy to be evident at an electron microscopic level and characterized by an increase in the number of vacuolated cells and widening of the intercellular spaces. Further, a topical allergen challenge enhanced the vacuolation of the epithelial cells. These results suggest that epithelial damage is a causative factor in the nasal hyperreactivity of nasal allergy.

REFERENCES

1. Connell JT. Quantitative intranasal pollen challenges: the priming effect in allergic rhinitis. J Allergy 1969;43:33-44.
2. Konno A, Togawa K, Nishihira S. Seasonal variation of sensitivity of nasal mucosa in pollinosis. Arch Otorhinolaryngol 1981;232:253-61.
3. Dunnill MS. The pathology of asthma, with special reference to changes in the bronchial mucosa. J Clin Pathol 1960; 13:27-33.
4. Filley WV, Holley KE, Kephart GM, Gleich GJ. Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. Lancet 1982;2:11-6.
5. Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. Am Rev Respir Dis 1985;131:599-606.
6. Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. Am Rev Respir Dis 1989;139:806-17.
7. Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma: an ultrastructural, quantitative study and correlation with hyperreactivity. Am Rev Respir Dis 1989;140:1745-53.
8. Ohashi Y, Motojima S, Fukuda T, Makino S. Airway hyperresponsiveness, increased intercellular spaces of bronchial epithelium, and increased infiltration of eosinophils and lymphocytes in bronchial mucosa in asthma. Am Rev Respir Dis 1992;145:1469-76.
9. Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hyperreactivity. J Allergy Clin Immunol 1988;81:776-81.
10. Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. J Allergy Clin Immunol 1989;83:1013-26.
11. Toppozada HH, Talaat MA. Human nasal epithelium and cellular elements in chronic allergic rhinitis. Electron-microscopic study. ORL J Otorhinolaryngol Relat Spec 1975;37:333-43.
12. Kawabori S, Nakamura A, Kanai N. Tissue density and state of activation of eosinophils in the nasal mucosa of allergic and nonallergic rhinopathic patients. Allergy 1994;49:81-5.
13. Kobayashi N, Dezawa M, Nagata H, Yuasa S, Konno A. Immunohistochemical study of E-cadherin and ZO-1 in allergic nasal epithelium of the guinea pig. Int Arch Allergy Immunol 1998;116:196-205.
14. Buckle FG, Cohen AB. Nasal mucosal hyperpermeability to macromolecules in atopic rhinitis and extrinsic asthma. J Allergy Clin Immunol 1975;55:213-21.
15. Inagaki M, Sakakura Y, Itoh H, Ukai K, Miyoshi Y. Mac-

romolecular permeability of the tight junction of the human nasal mucosa. Rhinology 1985;23:213-21.

16. Ohkubo K, Baraniuk JN, Hohman RJ, et al. Human nasal mucosal neutral endopeptidase (NEP): location, quantitation, and secretion. Am J Respir Cell Mol Biol 1993;9:557-67.

17. Ohkubo K, Okuda M, Kaliner MA. Immunological localization of neuropeptide-degrading enzymes in the nasal mucosa. Rhinology 1994;32:130-3.

18. Konno A, Nagata H, Nomoto M, Motosugi H, Terada N. Role of capsaicin-sensitive trigeminal nerves in development of hyperreactive nasal symptoms in guinea pig model of nasal allergy. Ann Otol Rhinol Laryngol 1995;104:730-5.

≈

NINTH INTERNATIONAL FACIAL NERVE SYMPOSIUM

The Ninth International Facial Nerve Symposium will be held July 29 to August 1, 2001, in San Francisco, California. For information, contact Office of Continuing Medical Education, University of California, San Francisco; telephone: (415) 476-5808; fax: (415) 502-1795; e-mail: info@ocme.ucsf.edu.

Ann Otol Rhinol Laryngol 110:2001

MUCOSA-ASSOCIATED LYMPHOID TISSUE IN MIDDLE EAR AND EUSTACHIAN TUBE

MASAMI KAMIMURA, MD, DMSC
ISAMU SANDO, MD, DMSC
CAREY D. BALABAN, PHD
SHIN-ICHI HAGINOMORI, MD, DMSC
PITTSBURGH, PENNSYLVANIA

The presence of mucosa-associated lymphoid tissue (MALT) was investigated histopathologically in every 20th section from 99 vertically cut, celloidin-embedded temporal bone–eustachian tube (ET) specimens. Among specimens from infants and children between 1 month and 7 years of age, MALT was found in 22 of 44 (50%). However, in 26 adults over 18 years of age, MALT was found in only 2 specimens (7.7%), a significantly lower incidence than that in infants and children. Moreover, MALT did not appear in any of the 21 neonates under the age of 1 month. All 99 specimens were classified into 2 groups: 41 specimens with otitis media (OM) and 58 specimens without OM. The presence of MALT was significantly higher in specimens with OM (43.9%) than in specimens without OM (13.8%). Mucosa-associated lymphoid tissue was found in the ET, middle ear, and mastoid process in 18 specimens (43.9%), 5 specimens (12.2%), and 1 specimen (2.4%) with OM, respectively, and in 8 (13.8%), 0, and 0 specimens without OM. In regard to the distribution of MALT, it occurred more frequently in the pharyngeal half of the cartilaginous portion of the ET than in the rest of the ET, middle ear, and mastoid; the presence was significantly greater in the inferior half of the cartilaginous portion of the ET than in the superior half. Inflammatory cell infiltration in the cartilaginous and bony portions of the ET was significantly greater in specimens with OM than in specimens without OM with no MALT. However, even in some specimens without OM, inflammatory cells were found in the ET, particularly in the pharyngeal half of the cartilaginous portion of the ET. These findings suggest that MALT has a close relationship to OM and that it may be a local response to repeated infection.

KEY WORDS — eustachian tube, histopathology, mucosa-associated lymphoid tissue, otitis media, temporal bone.

INTRODUCTION

Otitis media (OM) with effusion is one of the most frequently encountered conditions in children. It is believed that the eustachian tube (ET) plays an important role in the pathophysiology of this disease. However, there have been few histopathologic studies of the correlation between inflammation of the middle ear (ME) and inflammation of the ET.[1] It is known that children with OM display inflammatory cells and mucosa-associated lymphoid tissue (MALT) in the ME and ET mucosa.[2] Van der Baan et al[3] reported that MALT was found in 18 of 21 biopsy specimens (86%) obtained from the ME mucosa of children with OM. In 14 cases of children with OM, Matsune et al[2] found MALT in the ET in 9 cases (64%) and in the ME in 3 cases (21%).

Mucosa-associated lymphoid tissue is characterized histologically by rounded aggregations of lymphocytes in the lamina propria, usually with a germinal center. The number and size of lymphoid follicles vary among each of the tissues. It is assumed that MALT is closely associated with immunologic defense mechanisms and that MALT supplies cells committed to the production of IgA.[4]

This histopathologic study examined the presence of MALT in the ET, ME, and mastoid process in specimens with OM and in control specimens (no OM). The presence of inflammatory cells was also examined.

MATERIALS AND METHODS

This report is based on analyses of 99 human temporal bone–ET specimens obtained at autopsy from 99 individuals whose ages ranged from 1 day to 86 years (mean, 16.2 years). Of those 99 specimens, 58 showed no evidence of OM. The specimens were fixed in 10% formalin, decalcified by 5% trichloroacetic acid, dehydrated in graded ethanol, and embedded in celloidin. They were cut in a plane perpendicular to the long axis of the ET (ie, vertical plane) at a 30-µm thickness. Every 10th or 20th section was stained routinely with hematoxylin and eosin[5] for histopathologic analysis.

To investigate the location and the amount of MALT, the ET was divided into the pharyngeal half of the cartilaginous portion, the tympanic half of the cartilaginous portion, and the bony portion. Also, the cartilaginous portion of the ET was divided into su-

---

From the Elizabeth McCullough Knowles Otopathology Laboratory, Division of Otopathology, Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. This study was supported by research grant R01DC00123-22 from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders.

CORRESPONDENCE — Isamu Sando, MD, DMSc, Division of Otopathology, Dept of Otolaryngology, University of Pittsburgh School of Medicine, Eye and Ear Institute Building, Suite 153, 203 Lothrop St, Pittsburgh, PA 15213.

TABLE 1. PRESENCE OF MALT IN ET, MIDDLE EAR, AND MASTOID PROCESS

Age	*Sex*	*OM*	*C1*	*C2*	*B*	*ME*	*Mastoid*
1 mo	F	+	++	++	++	+++	+++
1 y	F	+	+++	+++	+++	+++	–
2 y	M	+	+++	+	+	+	–
2 y	F	+	++	++	+	+	–
5 y	F	+	+++	+	–	–	–
10 y	M	+	++	++	–	–	–
6 mo	F	+	+	+	–	–	–
8 mo	F	+	+	+	–	–	–
1 mo	F	+	+	–	+	+++	–
3 y	M	+	+++	–	–	–	–
1 y	M	+	++	–	–	–	–
2 y	M	+	++	–	–	–	–
4 mo	M	+	+	–	–	–	–
5 mo	M	+	+	–	–	–	–
9 mo	M	+	+	–	–	–	–
1 y	F	+	+	–	–	–	–
1 y	F	+	+	–	–	–	–
71 y	F	+	+	–	–	–	–
2 y	F	–	++	+	–	–	–
3 mo	F	–	+++	–	–	–	–
5 mo	F	–	+++	–	–	–	–
12 y	M	–	+++	–	–	–	–
1 y	F	–	+	–	–	–	–
3 y	F	–	+	–	–	–	–
5 y	M	–	+	–	–	–	–
19 y	F	–	+	–	–	–	–

OM — otitis media; C1 — pharyngeal half of cartilaginous portion of eustachian tube (ET); C2 — tympanic half of cartilaginous portion of ET; B — bony portion of ET; ME — middle ear; +++ — 3 or more mucosa-associated lymphoid tissue (MALT) follicles per section; ++ — 2 MALT follicles per section; + — 1 MALT follicle per section; – — no MALT follicles.

perior and inferior halves in each vertical section. Every 10th vertical section for the ME and mastoid and every 20th section for each of the ET portions described above was examined for the numbers of MALT follicles on the section, by means of the following grading system: 3 or more MALT follicles per section, 2 MALT follicles per section, 1 MALT follicle per section, or no MALT follicles.

Evidence of OM was defined by the presence of histopathologically confirmed inflammatory cell infiltration in the ME mucosa or ME cavity. The histopathologically defined cases of OM included not only cases of clinically diagnosed OM,[3,6] but also cases of "silent" OM, which have histopathologic evidence of ME inflammation but no clinical signs such as otorrhea or perforation.[7] We excluded cases with evidence of inflammation of the ET but no inflammation of the ME or mastoid. We defined normal specimens (without OM) by the absence of histopathologically identified inflammatory cell infiltration in the ME mucosa and ME cavity. For example, a specimen with inflammation of the ET only would be a normal specimen (without OM). The clinical records of these subjects were consistent with the assumption that these normal subjects (without OM) did not have a history of recurrent OM.

Classification of ME effusion (MEE) was made into the following 3 types: serous, a thin, watery fluid; purulent, a pus-like fluid; and mucoid, a thick, viscid fluid with mucous strands.[8,9]

The presence of inflammatory cells in the ET mucosa and lumen in each section was examined to investigate the correlation between OM and inflammation in the ET. If inflammatory cell infiltration was shown in both the ET mucosa and the lumen, the specimen was rated as positive.

The presence of MALT in the ET as a function of age and the inflammatory cell distribution in the ET as a function of the presence of OM were examined with Fisher's exact test.

RESULTS

Mucosa-associated lymphoid tissue was observed in the ET in 26 of the 99 temporal bones (Table 1). There was a greater presence of MALT in the pharyngeal half than in the tympanic half of the cartilaginous portion of the ET, ME, or mastoid ($p < .01$, Fisher's exact test). Every case with MALT in the mastoid also had MALT in the ME and ET; all cases with MALT in the ME also had MALT in the ET (Fig 1). The locations of MALT in the superior and inferior halves of the cartilaginous portion of the ET are summarized in Table 2. There was a significantly greater prevalance of MALT in the inferior half than in the superior half of the cartilaginous portion of the ET ($p < .01$, Fisher's exact test). Only 1 case showed MALT in only the superior half of the cartilaginous portion of the ET.

The prevalence of MALT in the ET among the 99 cases as a function of the age at death is shown in Table 3. Mucosa-associated lymphoid tissue did not appear in any of the 21 neonates (younger than 1 month of age). Among the infants and children between 1 month and 7 years old, MALT was found in 22 of the 44 specimens (50%) — an incidence that was significantly higher than that in adults ($p < .01$, Fisher's exact test) — and there were significant differences among some ages ($p < .01$, Fisher's exact test; Table 3). In adults, MALT was found in only 2 cases (7.7%): 1 of 9 subjects with OM and 1 of 17 subjects without OM. The presence of MALT in adults with OM versus adults with no evidence of OM was significantly elevated ($p < .01$, Fisher's ex-

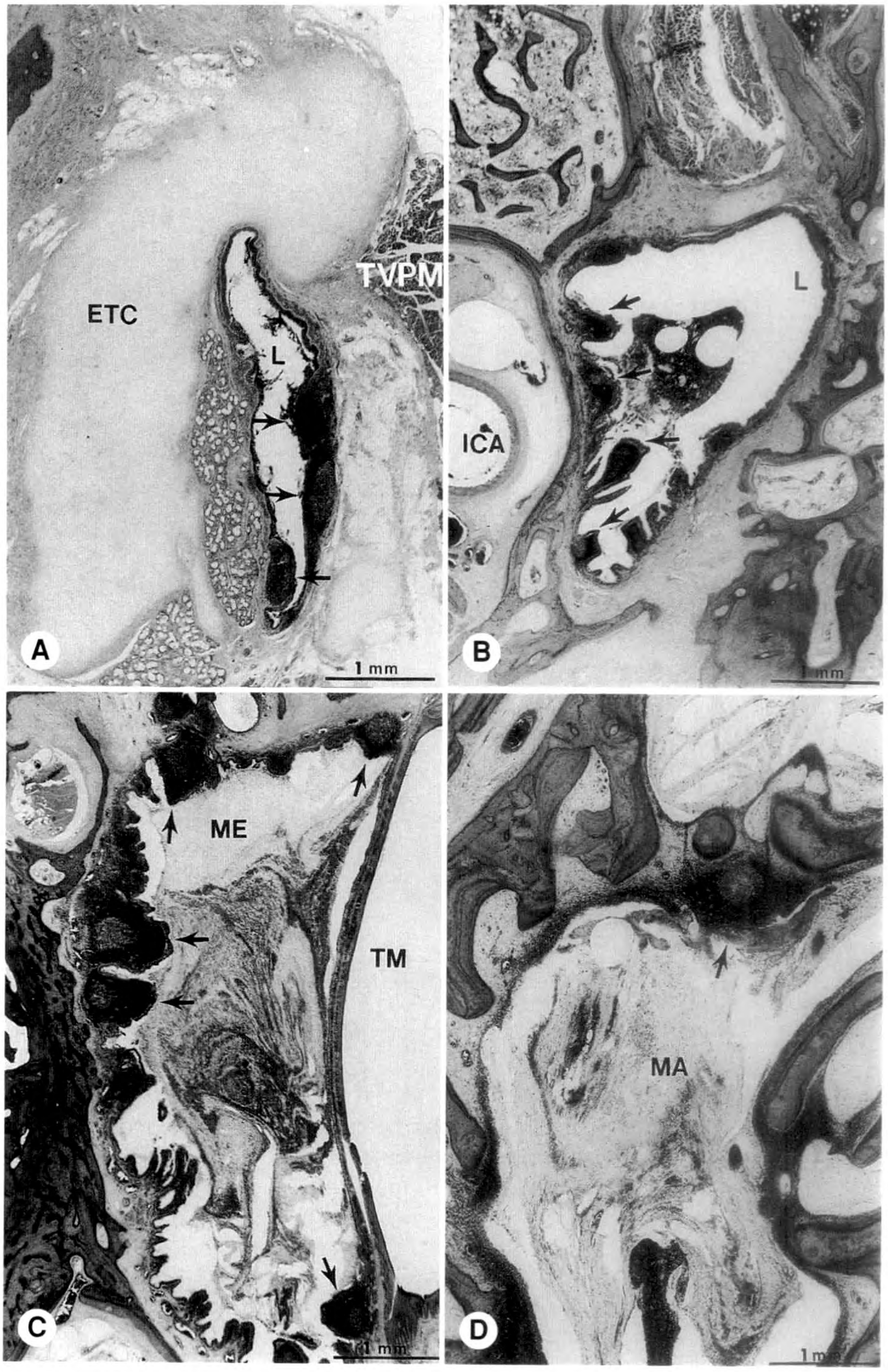


Fig 1. Temporal bone of 1-year-old infant girl with otitis media demonstrates mucosa-associated lymphoid tissue (arrows) in 4 different portions (H & E). A) Pharyngeal half of cartilaginous portion of eustachian tube (ET). ETC — ET cartilage, L — ET lumen, TVPM — tensor veli palatini muscle. B) Bony portion of ET. ICA — internal carotid artery, L — ET lumen. C) Middle ear. ME — middle ear cavity, TM — tympanic membrane. D) Mastoid antrum (MA).

act test). Among the cases of OM, there was no significant difference in the presence of MALT as a function of the type of MEE. We found MALT in 6 of the 19 specimens (31.6%) with serous MEE, in 2 of the 5 specimens (40.0%) with purulent MEE, and in 10 of the 17 specimens (58.8%) with mucoid MEE.

The relationship between inflammatory cell infiltration (Fig 2), the presence of MALT, and the presence of OM is shown in Table 4. In the OM cases, the presence of inflammatory cells in the ET mucosa or lumen was not affected by the presence of MALT. In the control specimens, there was a higher prevalence of infiltration in the cartilaginous ET for specimens with MALT in the ET (Fisher's exact test, $p < .05$ for each comparison), but no difference in prevalence for the bony segment of the ET.

DISCUSSION

Data from 26 temporal bone–ET specimens confirmed that there was a significant association be-

TABLE 2. MALT IN CARTILAGINOUS PORTION OF ET

Age	*Sex*	*OM*	*Sup*	*Inf*
1 mo	F	+		*
1 mo	F	+		*
4 mo	M	+		*
5 mo	M	+		*
6 mo	F	+		*
8 mo	F	+		*
9 mo	M	+		*
1 y	M	+		*
1 y	F	+		*
1 y	F	+	*	
1 y	F	+	*	*
2 y	M	+	*	*
2 y	F	+	*	*
2 y	M	+		*
3 y	M	+		*
5 y	F	+		*
10 y	M	+		*
71 y	F	+		*
3 mo	F	–		*
5 mo	F	–		*
1 y	F	–		*
2 y	F	–		*
3 y	F	–		*
5 y	M	–	*	*
12 y	M	–	*	*
19 y	F	–		*

Sup — superior half of cartilaginous portion of ET; Inf — inferior half of cartilaginous portion of ET; +/– — presence or absence of otitis media; * — presence of MALT.

tween MALT and OM. The presence of MALT was greatest in the ET, less in the ME, and least in the mastoid antrum. All cases with MALT in the ME also displayed MALT in the ET. Furthermore, MALT was present particularly in the inferior portion of the pharyngeal aspect of the cartilaginous portion of the ET. These findings suggest that when the inflammation occurs in the epipharynx, MALT may form initially in the pharyngeal half of the cartilaginous portion of the ET. We further suggest that MALT may proliferate toward the ME with recurrent infections (and inflammatory responses), and also that MALT remains after inflammation subsides.

TABLE 3. PREVALENCE OF MALT IN ET AS FUNCTION OF AGE

	Without OM		*With OM*		*Total*	
Age	*No.*	*%*	*No.*	*%*	*No.*	*%*
0 to 4 wk	0/18	0	0/2	0	0/20	0
1 mo to 7 y	6/18	33.3	16/26	61.5	22/44	50.0
8 to 17 y	1/5	20.0	1/4	25.0	2/9	22.2
18 to 88 y	1/17	5.9	1/9	11.1	2/26	7.7
Total	8/58	13.8	18/41	43.9	26/99	26.3

$p < .01$ (Fisher's exact test) for 0 to 4 weeks versus 1 month to 7 years; 1 month to 7 years versus 18 to 88 years; and total cases with OM versus those without.

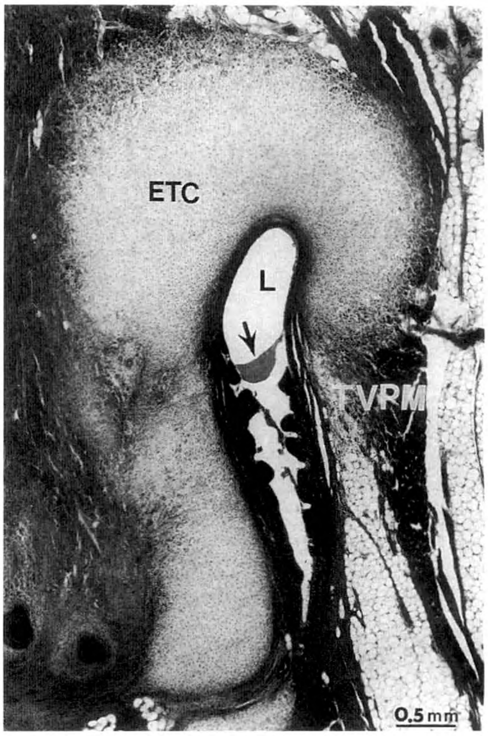


Fig 2. Temporal bone of 1-year-old infant girl with otitis media demonstrates inflammatory cell infiltration (arrow) in tympanic half of cartilaginous portion of eustachian tube lumen (L) and mucosa (H & E). ETC — eustachian tube cartilage, TVPM — tensor veli palatini muscle.

Both the presence of MALT in the ET and the similarities of lymphocyte populations in MALT and bronchus-associated lymphoid tissue[4,10] may indicate that the ET has mucosa that acts as an immune defense system similar to that of the respiratory system. The relative abundance of MALT within deep mucosal folds[11] in the inferior half of the ET strongly resembles that in the bronchial mucosal folds, which are thought to be important for lung clearance.[12,13] Thus, MALT is associated with regions of the ET that are 1) encountered initially by bacteria entering the ET-ME space from the nasopharynx (and, hence, receive a relatively high exposure to these organisms) and 2) exposed to cleared bacteria and bacterial debris in mucus moving toward the pharyngeal orifice.

The distribution of MALT in the ET varied with age. No MALT was observed in specimens from the first postnatal month. The prevalence of MALT was greatest in subjects 1 to 2 years of age (particularly in cases with OM), but it declined with age. This peak in the occurrence of MALT corresponds with the time period when approximately 50% of infants have MEE that lasts at least 4 weeks.[14] However, the presence of MALT is markedly reduced after 7 years of age, when the incidence of OM is reduced.[15] Thus, a pro-

TABLE 4. INFLAMMATORY CELL DISTRIBUTION IN ET

	OM With MALT in ET or ET Mucosa or Lumen Infiltrate		*OM With Neither MALT in ET nor ET Mucosa or Lumen Infiltrate*		*Control With MALT in ET or ET Mucosa or Lumen Infiltrate*		*Control With Neither MALT in ET nor ET Mucosa or Lumen Infiltrate*	
	Yes	*No*	*Yes*	*No*	*Yes*	*No*	*Yes*	*No*
Pharyngeal half of cartilaginous ET	18	0	21	2	8	0	19	31
Tympanic half of cartilaginous ET	18	0	22	1	7	1	13	37
Bony portion of ET	18	0	22	1	5	3	13	37

liferation of MALT is likely to be a local response to repeated infection. Two expected consequences of this MALT proliferation are 1) a more rapid and vigorous immune response in the ET and ME and, hence, 2) more severe inflammation during OM. From this perspective, the apparent regression of MALT in adults (present in only 2 of 23 cases) may be a simple consequence of the reduced prevalence of OM.

Interestingly, 38% of the non-OM specimens without MALT had inflammatory cells in the pharyngeal half of the cartilaginous portion of the ET. However, inflammatory cell infiltration of the cartilaginous ET in cases of OM with or without MALT and non-OM specimens with MALT was greater than that in specimens with no OM and no MALT. These findings may indicate that inflammatory cell infiltration in the ET is associated with OM and that it is a precursor to or consequence of the presence of MALT in the ET and ME concurrent with OM.

ACKNOWLEDGMENTS — The authors thank Eugene N. Myers, MD, Professor and Chairman of the Department of Otolaryngology, University of Pittsburgh School of Medicine, and Charles D. Bluestone, MD, Director, Department of Pediatric Otolaryngology, Children's Hospital of Pittsburgh, for their encouragement to study this subject. We also thank Gloria J. Limetti, Technician, for her assistance in this study.

REFERENCES

1. Sadé J, Luntz M, Berger G. The eustachian tube profile in children. Adv Otorhinolaryngol 1998;39:18-36.

2. Matsune S, Takahashi H, Sando I. Mucosa-associated lymphoid tissue in middle ear and eustachian tube in children. Int J Pediatr Otorhinolaryngol 1996;34:229-36.

3. van der Baan S, Seldenrijk CA, Henzen-Logmans SC, Drexhage HA. Serous otitis media and immunological reactions in the middle ear mucosa. Acta Otolaryngol (Stockh) 1988;106: 428-34.

4. Sato A, Chida K, Iwata M, Hayakawa H. Study of bronchus-associated lymphoid tissue in patients with diffuse panbronchiolitis. Am Rev Respir Dis 1992;146:473-8.

5. Sando I, Doyle WJ, Okuno H, Takahara T, Kitajiri M, Coury WJ III. A method for the histopathological analysis of the temporal bone and the eustachian tube and its accessory structures. Ann Otol Rhinol Laryngol 1986;95:267-74.

6. Palva T, Taskinen E. Inflammatory cells in chronic middle ear disease. Acta Otolaryngol (Stockh) 1990;109:124-9.

7. Paparella MM, Meyerhoff WL, Goycoolea MV. Silent otitis media. Laryngoscope 1980;90:1089-98.

8. Paparella MM. Middle ear effusions: definitions and terminology. Ann Otol Rhinol Laryngol Suppl 1976;85(suppl 25): 8-11.

9. Senturia BH, Paparella MM, Lowery HW, et al. Modified Report of the Ad Hoc Committee on Definition and Classification of Otitis Media. Ann Otol Rhinol Laryngol Suppl 1980; 89(suppl 69):6-8.

10. Kamimura M, Balaban CD, Sando I, Ganbo T, Suzuki C. Cellular distribution of mucosa-associated lymphoid tissue with otitis media in children. Ann Otol Rhinol Laryngol 2000;109: 467-72.

11. Sudo M, Sando I. Developmental changes in folding of the human eustachian tube. Acta Otolaryngol (Stockh) 1996;116: 307-11.

12. Aoki H, Sando I, Takahashi H. Mucosal folds of eustachian tube in young children. Auris Nasus Larynx 1993;20:125-30.

13. Sando I, Takahashi H, Aoki H, Matsune S. Mucosal folds in human eustachian tube: a hypothesis regarding functional localization in the tube. Ann Otol Rhinol Laryngol 1993;102: 47-51.

14. Pelton SI, Shurin PA, Klein JO. Persistence of middle ear effusion after otitis media. Pediatr Res 1984;11:504.

15. Bluestone CD, Klein JO. Otitis media, atelectasis, and eustachian tube dysfunction. In: Bluestone CD, Stool SE, Kenna MA, eds. Pediatric otolaryngology. 3rd ed. Philadelphia, Pa: WB Saunders, 1996:388-582.

Ann Otol Rhinol Laryngol 110:2001

ADENOID CYSTIC CARCINOMA OF THE BASE OF THE TONGUE

ALI NAMAZIE, MD
SASSAN ALAVI, MD
ELLIOT ABEMAYOR, MD, PHD
THOMAS C. CALCATERRA, MD
KEITH E. BLACKWELL, MD
LOS ANGELES, CALIFORNIA

A retrospective review of 14 patients with adenoid cystic carcinoma of the tongue treated between 1955 and 1997 was performed. Treatment consisted of surgery (n = 2), radiotherapy (n = 2), chemotherapy (n = 1), or combination therapy (n = 9). The 2-, 5-, and 10-year absolute survival rates were 92%, 79%, and 63%, respectively. Seventy-five percent of the patients who died of cancer succumbed to distant metastases. However, long-term survival was common despite a high incidence of local and distant recurrence. The presence of positive surgical margins, the incidence of regional metastases, the incidence of perineural invasion, the initial stage of disease, and the eventual development of locoregional recurrence and distant metastases did not significantly alter the survival rate. Surgical extirpation combined with postoperative radiotherapy is advocated for the treatment of adenoid cystic carcinoma of the tongue. Given the indolent nature of this disease process, surgery should be directed toward conservation of speech and swallowing function.

KEY WORDS — adenoid cystic carcinoma, base of tongue, minor salivary gland.

INTRODUCTION

Adenoid cystic carcinoma (ACC) is the most common malignant tumor of minor salivary gland origin.[1] In the tongue, ACC frequently presents as an asymptomatic submucosal mass, making early recognition difficult. Thus, patients often present late in the course of their illness.[2] The proclivity of ACC to extend along perineural pathways is well documented.[3] Consequently, pain, numbness, and intracranial extension of disease are frequently noted.[4,5] Adenoid cystic carcinoma has a slow and unrelenting course, with delayed recurrence being common. Many authors have advocated complete surgical extirpation with wide margins for long-term benefit.[4] Radiotherapy has been used in the treatment of inoperable or locally recurrent disease with some success.[6] The relative rarity of this tumor has made it difficult to critically evaluate the effectiveness of either treatment method.

It is generally accepted that most glandular tumors of the tongue have a worse prognosis than those in other locations in the oral cavity and oropharynx.[7,8] This may be the result of extensive submucosal spread, the inaccessibility of the region, or different tumor biological characteristics.[2] Whether ACC of the tongue falls into this category remains unclear. In order to further clarify the natural history of this tumor, a 42-year experience with ACC of the tongue at the University of California at Los Angeles School of Medicine was reviewed. An analysis of the clinical findings, therapeutic methods, and treatment results is presented.

METHODS

The medical records from the UCLA Center for Health Sciences from 1955 to 1997 were reviewed. Sixteen patients with ACC of the tongue were identified from the computerized records of the Department of Surgical Pathology and the UCLA Tumor Registry. Two patients were seen only in consultation, with no follow-up available, and were excluded from further analysis. Data were obtained from the medical and tumor registry records. Information regarding presenting symptoms and therapy was available for 14 patients. Follow-up was available for 13 patients. One patient, who received only chemotherapy, was lost to follow-up after discharge. Survival data were calculated with Kaplan-Meier life tables. Prognostic variables were examined with a χ^2 test.

RESULTS

The patients ranged in age from 24 to 78 years, with a mean of 56 years. There were 4 men and 10 women, for a ratio of 1 to 2.5. The presenting symptoms included dysphagia, otalgia, odynophagia, neck mass, tongue mass, and chest pain (Table 1). Several patients presented with more than 1 complaint. In 50% of the patients, dysphagia was the presenting symptom. Pain was a presenting complaint in 36% of patients. The average duration of symptoms before presentation was 15 months, with a median duration of 7 months. There was 1 patient who suf-

---

From the Division of Head and Neck Surgery, University of California at Los Angeles School of Medicine, Los Angeles, California.

CORRESPONDENCE — Keith E. Blackwell, MD, Division of Head and Neck Surgery, Box 951624 UCLA Medical Center, 10833 Le Conte Ave, Los Angeles, CA 90095-1624.

TABLE 1. SYMPTOMS AT PRESENTATION

Symptom	*Incidence*
Dysphagia	50%
Otalgia	21%
Odynophagia	14%
Neck mass	7%
Tongue mass	7%
Chest pain	7%

fered from disease for 8 years before seeking treatment. Seventy-nine percent of patients had symptoms for 6 months or more before presentation. There was a history of smoking in 50% of the patients, and 36% had a history of regular alcohol use.

The site of origin within the tongue was identified in 12 cases. Seventy-one percent of tumors (10/14) were located at the base of the tongue. Tumor laterality was almost equal, with 5 presenting on the right side and 7 on the left side. Tongue mobility was impaired in 21% of patients, and 71% of patients presented with a completely submucosal lesion. The size of the tumor at the time of presentation ranged from 1.5 to 6.0 cm. The average size at presentation was 3.5 cm. A histopathologic diagnosis was available for 11 of the 14 patients. Five specimens were classified histologically as cribriform, 3 as tubular, and the remaining 3 as solid. Six of the 14 patients (43%) had palpable suspicious cervical lymphadenopathy at the time of presentation. All of these patients had histologically confirmed ACC involving the cervical lymphatics. On initial evaluation, 10 of the 14 cases (71%) were classified as stage III or stage IV (Table 2).

Treatment consisted of surgery alone (2 patients), radiotherapy alone (2 patients), chemotherapy alone (1 patient), or combined therapy (9 patients). In the combined therapy group, all patients underwent surgery and radiotherapy, and chemotherapy was also administered to 4 patients. One patient in the combined therapy group was treated with preoperative radiotherapy; the remaining 8 had postoperative radiotherapy. On final pathological review, 6 of the 11 patients who underwent surgery were noted to have perineural invasion (55%), and 5 patients had positive microscopic margins (45%).

Local recurrence was observed in 4 of 13 patients, with 1 patient having 2 separate local recurrences at intervals of 5 and 10 years after treatment. The average time to local recurrence was 137 months, with a median interval of 101 months. Of the patients with a local recurrence, 2 demonstrated a cribriform pattern, and 2 had a tubular pattern. None of the patients with a solid histologic classification suffered a recurrence ($p = .08$). Of the 11 patients treated surgically, 3 had local recurrences. All 3 patients had histologically positive margins and perineural invasion. The remaining patient with a local recurrence was treated only with irradiation. Of the 8 surgically treated patients without a local recurrence, 75% (6/8) had microscopically negative margins, and 63% (5/8) had no perineural invasion. Thus, the incidence of local recurrence was significantly altered by the status of the surgical margins ($p = .026$, $\chi^2 = 4.95$), and the effect of perineural invasion on local recurrence approached statistical significance ($p = .064$, $\chi^2 = 3.44$). Of note, the presence of palpable cervical adenopathy and initial tumor size did not significantly affect the incidence of locoregional recurrence. The distribution of the recurrences by treatment category is shown in Table 3.

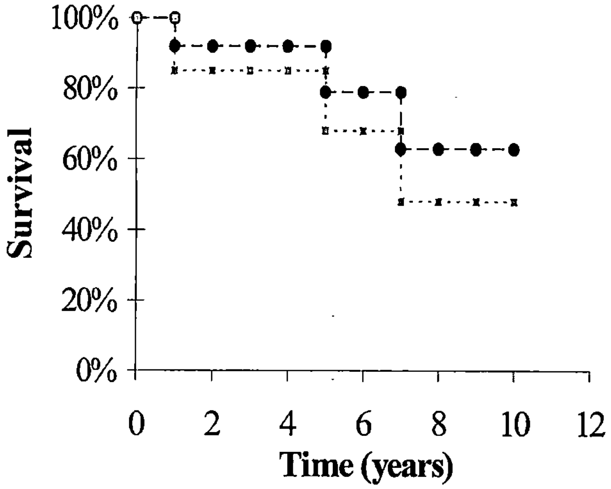
TABLE 2. STAGE AT PRESENTATION

Stage	*No. of Patients*	*%*
I	2	14.0
II	2	14.0
III	5	35.7
IV	5	35.7

TABLE 3. INCIDENCE OF LOCAL RECURRENCE AND DISTANT METASTASIS BY TREATMENT METHOD

	Local Recurrence		*Distant Metastasis*	
Treatment Method	*No.*	*%*	*No.*	*%*
Surgery only (n = 2)	1	50	1	50
Irradiation only (n = 2)	1	50	1	50
Combined treatment (n = 9)	2	22	5	56
Total (n = 13)	4	31	7	54

Of the 6 patients with cervical metastases, 5 underwent regional lymphadenectomy at the time of resection of the primary tumor. The remaining patient received radiotherapy to both the primary site and the cervical lymphatics. Fifty percent of cases (4/8) without cervical metastases were identified as stage III and IV. Only 1 patient with cervical metastasis died of disease (17%). On the other hand, 3 of the 7 patients without cervical metastases died of disease (43%). Distant metastases were found in 3 patients with cervical lymphatic involvement (50%). Fifty-seven percent of patients without cervical lymphatic involvement developed distant metastases (4/7). Of the 5 patients who underwent neck dissection, 3 received postoperative radiotherapy. All remained alive at the end of the follow-up period. The average survival time for patients with neck metastases was 82 months, whereas the average survival time for patients without neck disease was 124 months. Whether or not a patient received radiotherapy after neck dissection did not significantly impact survival time. Locoregional recurrence and distant metastasis were



Overall cumulative survival data for all patients (black circles) compared to those with distant metastases (gray boxes).

seen in 1 of the 3 patients who received radiotherapy after neck dissection. Of the 2 patients who only underwent neck dissection, locoregional recurrence and distant metastasis were seen in 1.

Seven patients (54%) developed distant metastases: 6 in the lungs and 1 in the cavernous sinus. Table 3 illustrates the incidence of distant metastases by initial therapeutic method. Of the patients with pulmonary metastases, 3 had multiple pulmonary nodules, and 3 presented with solitary nodules. Two patients with multiple pulmonary metastases received no treatment, and 1 was treated with chemotherapy. These 3 patients died of disease at 9, 59, and 75 months. All patients with solitary pulmonary metastases underwent excision of their pulmonary lesions, and 2 received chemotherapy postoperatively. They were alive at 65, 235, and 246 months. Histopathologic diagnosis was available for 5 patients with distant metastases. Two patients had a solid pattern, 2 had a cribriform pattern, and 1 had a tubular pattern. Thus, 67% of patients (2/3) with a solid histologic appearance developed distant metastases, whereas only 33% of patients (1/3) with a tubular histology had distal recurrences. This difference was not statistically significant. The average time for the development of distant metastases was 54 months, with a median of 21 months. The 2-, 5-, and 10-year survival rates for patients with distant metastases were 85%, 68%, and 48%, respectively (see Figure).

Of the patients with evidence of perineural invasion at the time of surgery, 83% (5/6) went on to develop distant metastases. In contrast, only 20% (1/5) of patients without evidence of perineural invasion demonstrated distant metastases ($p = .036$, $\chi^2 = 3.6$). The cases with distant metastases were staged at presentation as follows: 2 cases were stage II, 4 cases were stage 3, and 1 case was stage IV. The presence of tumor at the surgical margins, palpable cervical adenopathy, tumor stage, and locoregional recurrence did not significantly affect the incidence of distant metastases.

TABLE 4. ANALYSIS OF SEVERAL PROGNOSTIC VARIABLES

	Overall Survival Rate (%)		
Variable	*2 y*	*5 y*	*10 y*
Distant metastasis			
Present	83	60	40
Absent	100	100	100
p	>.05	>.05	>.05
Surgical margins			
Present	100	75	75
Absent	100	100	0
p	>.05	>.05	>.05
Cervical adenopathy			
N+	75	67	67
N0	100	75	67
p	>.05	>.05	>.05
T stage			
T3 + T4	80	67	67
T1 + T2	100	75	67
p	>.05	>.05	>.05
Recurrence			
Present	100	100	100
Absent	100	75	50
p	>.05	>.05	>.05
Neural invasion			
Present	100	75	67
Absent	100	100	50
p	>.05	>.05	>.05

The 2-year absolute survival rate for the entire group was 92%, and the 5-year and 10-year absolute survival rates were 79% and 63%, respectively (see Figure). Of the 3 patients who died during the study period, 2 had a solid histologic pattern, 1 had a cribriform pattern, and none had a tubular appearance. Thus, 67% of patients with a solid histologic pattern died of disease, compared to none with a tubular pattern ($p = .08$). Four patients survived beyond 10 years, with only 1 patient remaining free of disease for the duration of the follow-up period. Two other long-term survivors were alive with distant metastases, and 1 long-term survivor was alive with recurrent locoregional disease at the end of the follow-up period. The 2 patients who survived beyond 10 years with pulmonary metastases each had their metastatic lesions excised twice. Of the 43% of patients (3/7) with distant metastasis who died of their disease, none received therapy for their metastases. These patients died at 4, 8, and 41 months after diagnosis of distant metastases (average, 18 months). Survival time was not significantly altered by the incidence of distant metastases, the status of the surgical margins, the incidence of cervical adenopathy, the tumor stage, the presence of perineural invasion, or the incidence of locoregional recurrence (Table 4).

DISCUSSION

The upper aerodigestive tract contains a large number of minor salivary glands. These are present on the buccal and labial mucosa, the floor of the mouth, the tongue, the hard and soft palates, the pharynx, and the larynx. Approximately 450 to 750 minor salivary glands are found in the oral cavity and pharynx.[9] Minor and major salivary gland tumors share many histologic characteristics. However, they differ in distribution and biological behavior. Whereas minor salivary gland tumors are approximately 10 times less frequent than major salivary gland tumors, they have a higher incidence of malignancy.[10,11] In keeping with the distribution of minor salivary glands within the oral cavity and pharynx, the most common site of malignant minor salivary gland tumors is the hard palate, with the base of the tongue being the second most common location (approximately 20%).[11] In the base of the tongue, up to 96% of salivary gland tumors are malignant.[12,13] Adenoid cystic carcinoma accounts for more than 30% of malignant tumors of the minor salivary glands of the base of the tongue.[14]

Adenoid cystic carcinoma is usually monolobular and nonencapsulated or partially encapsulated. There is a variable histologic relationship between the neoplastic epithelial component of the lesion and the surrounding stroma. The stroma may consist of a mucinous myxoid interstitium or a hyaline eosinophilic material. The epithelial component generally consists of uniform basaloid cells with monotonous nuclei and scant cytoplasm. The canaliculi and the peripheral duct system of the salivary glands are the most likely sites of origin of this neoplasm.[1] Three histologic types of ACC have been identified: tubular, cribriform, and solid. Some authors have described a correlation between histology and clinical behavior.[15] Predominantly solid neoplasms appear to have the worst prognosis, whereas the tubular pattern denotes a less aggressive tumor. In contrast, others have found that the clinical stage of the tumor offers a more reliable prognostic guide.[16]

In this retrospective review, given the small sample size (albeit larger than most), no strong conclusions are possible. There was a female predominance of 2.5 to 1, and the mean age at presentation was in the sixth decade. Clinically, dysphagia was the most frequent presenting complaint. Pain was less commonly noted on initial evaluation (36%). Other presenting signs included otalgia and odynophagia. A palpable mass was observed in 29%. These patient characteristics and presenting symptoms are all consistent with the literature.[2,3,7,12,14,17] Tumors at the base of the tongue frequently reach a large size before becoming symptomatic and can be endured for long periods of time.[2,4,17,18] In this study, 71% of patients presented with a submucosal mass (mean size, 3.5 cm; Table 2). Goepfert et al[2] reported that 75% of cases of ACC of the base of the tongue were staged as T3 or T4 at the time of presentation.

The treatment of ACC remains controversial. Traditional external beam radiotherapy as a primary treatment method has been deemed inadequate.[8] Most authors agree that ACC is radiosensitive but not radiocurable.[4] More recently, neutron radiotherapy has been reported to be more effective than conventional irradiation in the treatment of surgically unresectable salivary gland tumors.[6] This finding may be due to 2 factors: 1) neutrons are more densely ionizing than photons, causing a greater percentage of double-stranded DNA damage within tumor cells,[19] and 2) neutrons are less dependent on the presence of oxygen within a tumor to achieve a therapeutic effect.[20] Whether neutron beam therapy alone will prove useful as a primary mode of treatment remains to be studied. Traditionally, indications for the use of adjuvant radiotherapy have included marked perineural invasion, high-grade tumors, and microscopic disease near or beyond the margins of resection.[2] Because we had only 2 patients who received primary radiotherapy, no conclusions can be reached from this study regarding the efficacy of this therapeutic approach.

This tumor is well known for its propensity to spread along perineural spaces.[2] Luna et al[3] found that of 33 patients with ACC of the minor salivary glands, 17 had evidence of perineural invasion.[3] Intracranial extension of disease via cranial nerves has been reported, particularly along the trigeminal and glossopharyngeal nerves.[21] Our data indicated a 55% incidence of perineural invasion. In the current study, the presence of perineural invasion had a significant effect on the development of distant metastases, and the impact of perineural invasion on local recurrence approached significance. The propensity toward local tumor extension by perineural invasion is a deterrent to successful management of ACC.[1] Despite aggressive surgical resections, 5 of 11 patients in our series had microscopically positive margins.

A high rate of distant metastasis may also contribute to the poor prognosis in some patients with ACC of the tongue.[8] Fifty-four percent of patients in this study eventually developed distant metastases. As compared to the tubular pattern, patients with a solid histologic pattern had a higher incidence of distant metastasis and death. In the literature, the metastatic potential of lingual ACC has been reported to range from 23% to 44%.[3,7,8,17] In the current study, the highest rate of distant metastasis was seen in the combined therapy group (Table 3). However, the metastatic rate was not significantly different among the 3 treatment methods. In the combined therapy group, the patients who received chemotherapy had a higher incidence of distant metastases (3/4) than the patients who did not receive chemotherapy (2/5). This may demonstrate the proclivity of ACC to metastasize regardless of the treatment approach. Further, the patients who received chemotherapy were most likely to be those in whom the primary lesion appeared more aggressive. Interestingly, the median time from presentation to the development of distant metastasis was much shorter than the median time to local recurrence. This finding implies that many patients may have occult distant metastases at the time of initial presentation, or alternatively, patients with distant metastases may have more biologically active tumors.

In the current series, 43% of patients presented with neck masses that were pathologically confirmed as metastatic ACC. This is higher than the metastatic potential generally cited in the literature for this tumor. Potential explanations are the rich lymphatic drainage of the base of the tongue or the late stage of presentation of ACC of this region. Interestingly, neither the incidence of distant metastases nor the overall survival rate was significantly affected by the status of the cervical lymph nodes. In the patients who underwent neck dissection and radiotherapy, a lower likelihood of locoregional recurrence and distant metastasis was noted as compared to those who underwent only neck dissection.

In this study, 6 prognostic variables were analyzed for effect on overall survival rate (Table 4). None of these variables had any significant impact on overall survival rate. In analyzing patients with ACC of minor salivary gland origin, Fu et al[22] have reported 5- and 10-year actuarial survival rates of 66% and 46%, respectively. Sur et al[5] found overall survival rates of 38% and 29% at 5 and 10 years, respectively, in a series of 50 patients. Our data demonstrate an overall survival rate of 79% at 5 years and 63% at 10 years. This improved survival rate may be due to improved therapeutic methods at our institution. Alternatively, it may indicate that at 5 and 10 years, ACC of the tongue has a better prognosis than ACC elsewhere in the head and neck.

Surgical resection has been the primary mode of treatment for ACC. The extent of resection — radical versus limited — has been debated in the literature.[23] Adenoid cystic carcinoma involving the base of the tongue presents a unique clinical dilemma, because aggressive surgery in this region frequently has a profound adverse impact on speech and swallowing. Our data indicate that the presence of tumor at the surgical margins significantly affected the development of locoregional recurrence. However, the status of the surgical margins and the incidence of tumor recurrence had no impact on overall survival rate. As long-term survival is the rule even in the presence of locoregional or distant recurrence, it is appropriate to consider factors that affect the quality of life in determining the optimal therapeutic approach to patients with ACC of the tongue. Therefore, we advocate a surgical approach that is directed toward tumor excision with conservation of speech and swallowing function. In the base of tongue region, this may necessitate the acceptance of close surgical margins in order to preserve the supraglottic larynx and the function of at least 1 hypoglossal nerve. Death from local recurrence in the tongue base is very painful, and every attempt should be made to obtain free surgical margins without significant morbidity. Our larger experience in treating cancers of the minor salivary glands indicates that there is a beneficial role of postoperative radiotherapy in cases in which the surgical margins are close.[24] This concept is supported by previous reports that have shown a survival benefit with the use of combination therapy (surgery with conventional radiotherapy) in advanced-stage ACC.[25,26] Further, the combined therapy group had the lowest rate of local recurrence in the current series (Table 3), perhaps representing the efficacy of radiotherapy as an adjunct to surgery for the control of local disease. Postoperative radiotherapy is advocated for patients with advanced-stage disease, close surgical margins, or perineural invasion to decrease the chance of local recurrence. We believe that this approach offers the highest likelihood of achieving an acceptable quality-of-life outcome in a patient population that frequently demonstrates prolonged survival time after treatment despite a high incidence of recurrence.

Patients with ACC of the tongue are unlikely to be cured of their disease.[8] However, long-term survival is possible even with locoregional or distant disease. Of the patients who survived beyond 10 years in the current series, only 1 was free of disease at the time of death. The remaining long-term survivors suffered from locoregional recurrence or distant metastasis. Although none of the patients with locoregional recurrence died of their disease (0/4), 3 of the 7 patients with distant metastases eventually succumbed. These data demonstrate that death is more likely to result from distant metastasis than from locoregional recurrence. Luna et al[3] demonstrated that 10 of their 17 patients who died suffered from distant metastases, whereas only 4 of the 17 patients died of local recurrence. In the current study, the patients who survived longer than 10 years with distant metastases had surgical excision of their metastatic pulmonary lesions. In comparison, none of the patients who died of metastatic disease underwent treatment for these lesions. Consequently, it may be argued that surgical resection of a solitary pulmonary metastasis is worthwhile, as long as the primary lesion is controlled.

CONCLUSION

Adenoid cystic carcinoma of the tongue is a relatively uncommon disease. Our conclusions are based on a small series of patients treated over a long period of time. This tumor is difficult to eradicate by surgical excision. The incidence of local recurrence correlates significantly with the status of the surgical margins. The presence of perineural invasion correlates significantly with the occurrence of distant metastases, and the effect of perineural invasion on local recurrence approaches statistical significance. Death is most commonly due to distant metastases. However, long-term survival is common, despite a high incidence of local recurrence and distant metastasis. A surgical approach directed toward tumor excision with preservation of speech and swallowing function is appropriate for patients with ACC of the tongue. Radiotherapy after surgical resection is generally justified for the treatment of this tumor, and there may be a palliative role for surgical excision of metastatic pulmonary lesions. Because ACC may progress in a slow, relentless fashion, long-term follow-up is required for 20 years and beyond for the assessment of therapy results.

REFERENCES

1. Batsakis JG. Tumors of the major salivary glands. In: Batsakis JG, ed. Tumors of the head and neck: clinical and pathological considerations. Baltimore, Md: Williams and Wilkins, 1979:38-51.
2. Goepfert H, Giraldo AA, Byers R, Luna M. Salivary gland tumors of the base of the tongue. Arch Otolaryngol 1976;102: 391-5.
3. Luna MA, Stimson PG, Bardwill JM. Minor salivary gland tumors of the oral cavity: a review of 68 cases. Oral Surg Oral Med Oral Pathol 1968;25:71-86.
4. Clark SK, Yarington CT. Lingual malignant disease of minor salivary gland origin. Am J Otolaryngol 1980;1:181-5.
5. Sur RK, Donde B, Levin V, et al. Adenoid cystic carcinoma of the salivary glands: a review of 10 years. Laryngoscope 1997;107:1276-80.
6. Douglas JG, Laramore GE, Austin-Seymour M, et al. Neutron radiotherapy for adenoid cystic carcinoma of minor salivary glands. Int J Radiat Oncol Biol Phys 1996;36:87-93.
7. Roper PR, Wolf PF, Luna MA, Goepfert H. Malignant salivary gland tumors of the base of the tongue. South Med J 1987;80:605-8.
8. Kessler DJ, Mickel RA, Calcaterra TC. Malignant salivary gland tumors of the base of the tongue. Arch Otolaryngol 1985;111:664-6.
9. Orban B. Oral histology and embryology. 3rd ed. St Louis, Mo: CV Mosby, 1953.
10. Vellios F, Shafer W. Tumors of the intraoral accessory salivary glands. Surg Gynecol Obstet 1959;108:450-6.
11. Chaudhry AP, Vickers A, Gorlin RJ. Intraoral minor salivary gland tumors: an analysis of 1414 cases. Oral Surg Oral Med Oral Pathol 1964;14:1194-223.
12. Goldblatt LI, Ellis GL. Salivary gland tumors of the tongue. Cancer 1987;60:74-81.
13. Ruby SG, Kish JK. Oncocytic adenocarcinoma of minor salivary gland: an unusual glossal presentation of a minor salivary gland tumor. Pathol Res Pract 1996;192:856-64.
14. Spiro RH, Huvos AG, Strong EW. Adenoid cystic carcinoma of salivary origin. Am J Surg 1974;128:512-20.
15. Wenig BM. Neoplasms of the salivary glands. In: Wenig BM, ed. Atlas of head and neck pathology. Philadelphia, Pa: WB Saunders, 1993:311-3.
16. Spiro RH, Huvos AG, Strong EW. Adenoid cystic carcinoma: factors influencing survival. Am J Surg 1979;138:579-83.
17. Burbank PM, Dockerty MB, Devine KD. A clinicopathologic study of 43 cases of glandular tumors of the tongue. Surg Gynecol Obstet 1959;109:537-82.
18. McGregor IA, McGregor FM. Cancer of the face and mouth. 1st ed. London, England: Churchill Livingstone, 1986: 470-93.
19. Griffin TW. High linear energy transfer and heavy charged particles. In: Perez CA, Brady LW, eds. Principles and practice of radiation oncology. 2nd ed. Philadelphia, Pa: JB Lippincott, 1992:368-9.
20. Hall EJ. Radiobiology for the radiologist. 3rd ed. Philadelphia, Pa: JB Lippincott, 1988:107-77.
21. Bardwill JM, Reynold CT, Ibanez M, Luna MA. Report of 100 tumors of the minor salivary glands. Am J Surg 1966;112: 493-7.
22. Fu KK, Leibel SA, Levine ML, Friedlander LM, Bolus R, Philips TL. Carcinoma of the major and minor salivary glands. Cancer 1977;40:2882-90.
23. Casler JD, Conley JJ. Surgical management of adenoid cystic carcinoma in the parotid gland. Otolaryngol Head Neck Surg 1992;106:332-8.
24. Tran L, Sidrys J, Sadeghi A, Ellerbroek N, Hanson D, Parker RG. Salivary gland tumors of the oral cavity. Int J Radiat Oncol Biol Phys 1990;18:413-7.
25. Sadeghi A, Tran LM, Mark R, Sidrys J, Parker RG. Minor salivary gland tumors of the head and neck: treatment strategies and prognosis. Am J Clin Oncol 1993;16:3-8.
26. Spiro RH. Salivary neoplasms: overview of a 35-year experience with 2,807 patients. Head Neck Surg 1986;8:177-84.

Ann Otol Rhinol Laryngol 110:2001

IS PRESSURE DRESSING NECESSARY AFTER EAR SURGERY?

MARCO L. CASTELLI, MD, FRCS
PAOLO TAVORMINA, MD
DIEGO DI LISI, MD
ELENA CAPPELLARO, MD
PAOLO MARCATO, MD
ALBERTO SARTORIS, MD, PHD
TURIN, ITALY

Compressive bandages carry intrinsic risks and complications. The purpose of this study was to assess whether compressive head bandages are necessary after ear surgery to prevent complications such as hematoma or wound infection. A prospective, randomized, controlled study was conducted from August 1, 1993, to June 1, 1999. We randomly assigned 420 consecutive middle ear or mastoid operations to either a pressure bandage group or to a no–pressure bandage group. A careful follow-up was planned, and complications were recorded. In the pressure bandage group, 3 patients had bruising over the pinna and 70 patients had minor skin erythema when the dressing was removed. No patient had bruising or erythema in the no–pressure bandage group. No patient had hematoma, wound infection, or any other wound complication in either group. As a result of this study, we have decided to abandon the use of compression bandages after uncomplicated ear surgery.

KEY WORDS — complications, dressings, ear surgery.

INTRODUCTION

By tradition, postauricular or endaural surgical wounds in middle ear and mastoid operations are covered by pressure dressings. Pressure dressings are widely considered to be necessary to minimize the risk of postoperative hematoma formation, especially when surgical drains are not inserted. Pressure dressings, however, in the form of such cumbersome head bandages can cause severe discomfort and headache, being even worse tolerated in hot weather; they may be responsible for compressive, irritative, or allergic skin reactions; they slightly increase the cost and length of the surgical procedure. Moreover, dressings may delay inspection of the wound, with obvious potential risks to the patient.

A prospective, randomized, controlled study was designed to assess the need for pressure dressings after ear surgery.

MATERIALS AND METHODS

Four hundred twenty consecutive middle ear or mastoid operations were randomly assigned to either a pressure bandage group or a no–pressure bandage group. The study began on August 1, 1993, and ended on June 1, 1999.

No patient had bleeding disorders as checked by blood test screening (international normalized ratio and activated partial thromboplastin time). The surgical operations were as follows: 290 myringoplasties with or without ossiculoplasty, 20 canal wall–up tympanoplasties, 75 canal wall–down tympanoplasties, and 35 radical mastoidectomies. All operations were performed with the patient under hypotensive general anesthesia, and all ears were locally infiltrated postaurally and inside the meatus with 1:200,000 adrenaline. In all patients, we used a postauricular approach. Wound closure was achieved with interrupted subcutaneous sutures (chromic catgut or polyglactin) and interrupted or continuous skin sutures (silk).

There were 405 patients included in the study, as 10 patients were operated on bilaterally and 5 patients had to be reoperated. We randomly allocated 212 cases to the pressure bandage group (130 cases in women and 82 in men; age range, 17 to 80 years; mean, 44 years) and 208 cases to the no–pressure bandage group (129 cases in women and 79 in men; age range, 15 to 80 years; mean, 45 years). In the pressure bandage group, 150 operations were performed by senior surgeons and 62 by trainees under strict supervision; in the no–pressure bandage group, 100 operations were performed by senior surgeons and 108 by trainees.

The patients who did not have a compressive bandage were simply dressed with gauze swabs kept in place with adhesive tapes, without compression. This was believed to be useful in collecting the blood that sometimes leaked from the wound. The dressing was then removed on the first postoperative day. The patients who had compressive dressing had a circumferential head bandage with 5 gauze swabs (6 x 6 cm) and cotton wool over the auricle and the mastoid. A ribbon gauze tied over the eyebrow helped to keep the bandage in place. Patients kept this dressing for 3

From the First Department of Otorhinolaryngology, University of Turin, Turin, Italy.

CORRESPONDENCE — Marco L. Castelli, MD, FRCS, Via Dei Prati 1, Savigliano 12038, Italy.

days after the operation. The wound was inspected daily for complications during the hospital stay in both groups. All patients were discharged home on the fifth postoperative day, when the stitches were removed. The first outpatient appointment was scheduled for 3 weeks from the day of discharge from the hospital. All patients had postoperative antibiotic treatment systemically (amoxicillin 1 g plus clavulanic acid daily for a week, or erythromycin if allergic) and locally (antibiotic ear drops with steroids for 2 weeks).

RESULTS

In the pressure bandage group, 3 patients had bruising over the pinna that had disappeared by the third week with no treatment; when the dressing was removed, 70 patients had minor skin erythema that subsided within 24 hours; 25 patients had minor wound dehiscence at the fifth postoperative day that healed after 1 week with local care.

In the no–pressure bandage group, no patient had bruising or erythema, and 19 patients had minor wound dehiscence that soon resolved. Bruising ($p < .05$) and erythema ($p < .05$) were statistically related to the pressure bandage.

No patient had hematoma, wound infection, or any other wound complication in either group, with the exception of 1 keloid that later developed in the pressure bandage group.

DISCUSSION

Dressings are thought to be useful in protecting the wound, preventing contamination, stabilizing the wound, providing an optimal healing environment, and absorbing secretions.[1-4] However, early exposure of surgical wounds has become very popular in many surgical specialties, and many surgeons question the need of dressing the wound at all.[5-11] Protection from trauma by way of dressing was thought by Weiss[12] to be unnecessary on the surgical ward or for ambulatory clinic patients.

Many studies that have compared the infection rate in undressed and dressed wounds have not demonstrated any beneficial effect of dressings.[9,11-13] Many authors actually acknowledge that dressings carry a risk of morbidity.

Bandages may cause collection of blood or mucus, and the environment under a head dressing may promote bacterial proliferation.[13,14] No patients in our study suffered from infections, possibly because of the local and systemic antibiotic treatment.

Powell[15] in 1989 found that even in the postoperative management of the prominent ear, pressure dressings can cause necrosis. Skin reactions are common with head bandages and may be caused by pressure or by allergy. In our study, we only had bruising or skin erythema in the head bandage group.

The main reason for treating a patient with postoperative pressure dressing is often to avoid hematoma formation. Pressure is thought to be necessary because drains are often avoided in ear surgery. In none of our 420 cases were drains inserted, and not a single case of hematoma was recorded, despite the fact that in 208 cases the wound was not compressed.

CONCLUSION

Patients often complain of headaches and discomfort caused by head bandages; some studies that have investigated patients' attitudes to wound care have shown that most patients do not mind whether their wounds are covered with a dressing or not.[10] Moreover, when the pressure exerted by head bandages after ear surgery was measured, it was found that after the first hour, bandages had often lost the pressure believed to be necessary to prevent hematoma formation.[16] It is well known that dressings can delay inspection of the wound and can thereby prevent prompt treatment of local complications such as hemorrhages, infections, and skin reactions.

We have not demonstrated that leaving the wound without a pressure dressing does not increase the risk of postoperative bleeding, but we showed that provided intraoperative hemostasis is accurate, the risk of hematoma formation is minimal. As a result of this study, we have abandoned the use of compression bandages after uncomplicated ear surgery.

REFERENCES

1. Harkins HN. Wound healing. In: Moyer CA, Rhoads JE, Allen JG, Harkins HN, eds. Surgery: principles and practice. 3rd ed. Philadelphia, Pa: JB Lippincott, 1965:25-7.

2. MacLaren IF. Simplified wound care. J R Coll Surg Edinb 1963;9:61-5.

3. Norton LW. Trauma. In: Hill GJ II, ed. Outpatient surgery. Philadelphia, Pa: WB Saunders, 1973:77-83.

4. Wolcott MW. Dressing and bandages. In: Wolcott MW, ed. Ferguson's surgery of the ambulatory patient. 5th ed. Philadelphia, Pa: JB Lippincott, 1974:35-7.

5. Heifetz CJ, Lawrence MS, Richards FO. Comparison of wound healing with and without dressings. Arch Surg 1952;65:746-51.

6. Heifetz CJ, Richards FO, Lawrence MS. Wound healing without dressings. Arch Surg 1953;67:661-9.

7. Hermann RE, Flowers RS, Wasylenki EW. Early exposure in the management of the postoperative wound. Surg Gynecol Obstet 1965;120:503-6.

8. Kleitsch WP, Reiser HG. The open treatment of surgical wounds. Am J Surg 1954;88:609-12.

9. Palumbo LT, Monnig PJ, Wilkinson DE. Healing of clean surgical wounds of thorax and abdomen with or without dressings. JAMA 1956;160:553-5.

10. Persson M, Svenberg T, Poppen B. To dress or not dress surgical wounds? Patients' attitudes to wound care after major abdominal operations. Eur J Surg 1995;161:791-3.

11. Chrintz H, Vibits H, Cordtz TO, Harreby JS, Waaddegaard P, Larsen SO. Need for surgical wound dressing. Br J Surg 1989;76:204-5.

12. Weiss Y. Simplified management of operative wounds by early exposure. Int Surg 1983;68:237-40.

13. Rowe-Jones JM, Leighton SEJ. The value of head dressings for middle ear surgery. J Laryngol Otol 1993;107:17-9.

14. Marshall DA, Mertz PM, Eaglstein WH. Occlusive dressings. Does dressing type influence the growth of common bacterial pathogens? Arch Surg 1990;125:1136-9.

15. Powell BWEM. The value of head dressing in the postoperative management of the prominent ear. Br J Plast Surg 1989;42:692-4.

16. Hill J, Allan W, Malhan D, Williams ED. Pressure exerted by head bandages used in otological surgery. J Laryngol Otol 1993;107:1110-2.

◆

INTERNATIONAL HEARING AID CONFERENCE

International Hearing Aid Conference VI. Novel Processing and Fitting Strategies will be held in Iowa City, Iowa, June 7-9, 2001. For information, see web site www.medicine.uiowa.edu/otolaryngology/news/news, or contact Rich Tyler by telephone (319-356-2471) or e-mail (rich-tyler@uiowa.edu).

Ann Otol Rhinol Laryngol 110:2001

HYPERTROPHIC NEUROPATHY OF THE FACIAL NERVE

ROMAIN E. KANIA, MD
DOMINIQUE CAZALS-HATEM, MD
DIDIER BOUCCARA, MD
FRANÇOISE CYNA-GORSE, MD
FABRICE LISOVOSKI, MD, PHD
DOMINIQUE HÉNIN, MD
OLIVIER STERKERS, MD, PHD

CLICHY, FRANCE

Hypertrophic neuropathy is a peripheral nerve lesion that is histologically characterized by onion bulb formations around axons. This histologic picture, which is usually seen in generalized hypertrophic neuropathies, can occasionally be observed in single nerves as localized hypertrophic neuropathy. Cranial involvement of such localized hypertrophic neuropathy represents a very rare entity; only a few cases have been reported in the literature. We report the history of a progressive facial paralysis with a tumorous enlargement of the seventh cranial nerve that was clinically suspected of being a schwannoma. Pathological examination permitted the diagnosis of hypertrophic neuropathy.

KEY WORDS — cranial nerve disease, facial nerve, facial paralysis, hypertrophy.

INTRODUCTION

Hypertrophic neuropathy is described as a nerve enlargement, histologically characterized by onion bulb formations,[1-3] that results from concentric proliferation of Schwann cells around the axon. Hypertrophic neuropathy is usually diffuse and observed in chronic demyelinating neuropathies, such as hereditary sensorimotor neuropathies (Charcot-Marie-Tooth disease or Dejerine-Sottas disease) or acquired demyelinating neuropathies. Sometimes, single nerves are locally affected by this process of chronic demyelination-remyelination; such localized hypertrophic neuropathy (LHN) has rarely been described in brachial plexus or limb nerves.[1-4]

Cranial nerve involvement in hypertrophic neuropathy is very rare.[5-9] A few observations in the literature have described what was called LHN affecting the trigeminal nerve, the acoustic nerve, or the facial nerve. Actually, some of these cases were perineurioma, which is the main differential diagnosis of LHN.[4] This tumoral process is a proliferation of perineurial cells around the axon, leading to a similar onion bulb pattern, but with distinct immunohistochemical characteristics. Therefore, the confusion between these two entities was maintained as a result of insufficient histologic data.

We report here a well-documented case of focal hypertrophic neuropathy affecting a facial nerve lesion that was clinically considered an intratemporal schwannoma. The differential diagnosis, cause, and treatment of this hypertrophic neuropathy of the facial nerve are discussed.

CASE REPORT

A 44-year-old woman with a 9-month history of hypoacusis and progressive right facial palsy was referred to our clinic. She had no tinnitus or vertigo, no history of previous head trauma, no personal or familial history of neurologic disease, and no diabetes. She had never been to a tropical country.

Clinical examination confirmed a right facial paralysis staged as grade II according to the House and Brackmann facial nerve grading system.[10] A retrotympanic mass was seen in the posterosuperior area of the tympanic membrane. Facial hemispasm could be triggered by applying pressure to the tumor mass. The external auditory canals and the contralateral tympanic membrane were normal. The findings of neurologic examination and ophthalmoscopy were normal. She had no café au lait spots or subcutaneous nodules. The right parotid gland was normal.

An audiogram revealed pure tone thresholds ranging from 25 to 120 dB (air-bone gap, 5 to 20 dB) and speech discrimination alteration. The findings of tympanometry and electronystagmography and the auditory brain stem evoked responses were normal. Schirmer's test provoked symmetric lacrimal secretion, and the stapedial reflex was normal. Electroneurography showed slight denervation of the right facial muscles with a delayed eye-blink reflex.

From the Departments of Otorhinolaryngology–Head and Neck Surgery (Kania, Bouccara, Sterkers), Pathology (Cazals-Hatem), Radiology (Cyna-Gorse), and Neurology (Lisovoski), Assistance Publique–Hôpitaux de Paris, Hôpital Beaujon, and the Department of Pathology, Assistance Publique–Hôpitaux de Paris, Hôpital Bichat (Hénin), Faculté Xavier Bichat, University Paris VII, Clichy, France.

CORRESPONDENCE — Olivier Sterkers, MD, PhD, Dept of Otorhinolaryngology–Head and Neck Surgery, Hôpital Beaujon, AP-HP, Université Paris VII, 100 boulevard du Général Leclerc, 92118 Clichy Cedex, France.

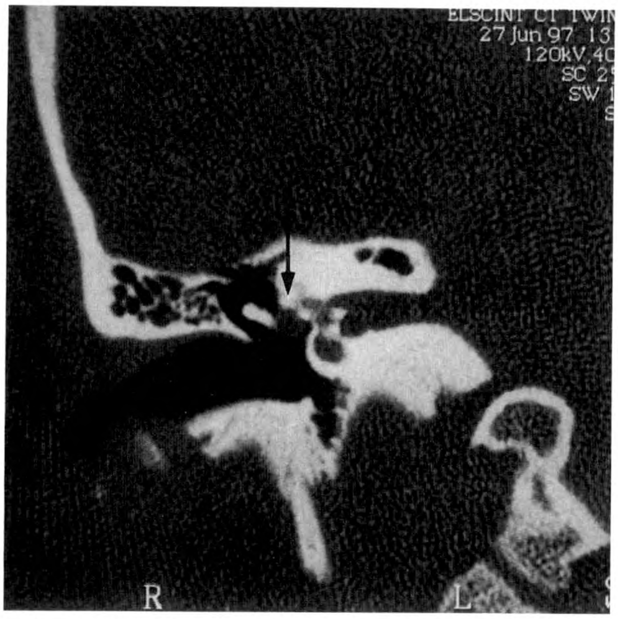


Fig 1. Coronal computed tomography (CT) scan shows regular enlargement of facial nerve's tympanic segment. Walls of facial canal have disappeared, whereas inner wall of tympanic cavity is normal. Tumor is in contact with incus and oval window (arrow).

A computed tomography scan revealed enlargement of the right facial nerve from the geniculate ganglion (Figs 1 and 2) to the stylomastoid foramen. A nodule was individualized in both mastoid and tympanic segments of the facial nerve. The stapes, incus, and oval window were found in contact with the nodule (Fig 1). Circumferential widening of the right fallopian canal was observed (Fig 3). The left facial nerve was normal.

Magnetic resonance imaging revealed slight and homogeneous gadolinium enhancement of the geniculate ganglion and tympanic and mastoid segments of the right facial nerve (Fig 4). On T1- and T2-weighted images, the lesion was more intense than muscle. The parotid gland was free of lesion. A diagnosis of intratemporal schwannoma of the facial nerve extending from the geniculate ganglion to the stylomastoid foramen was suspected.

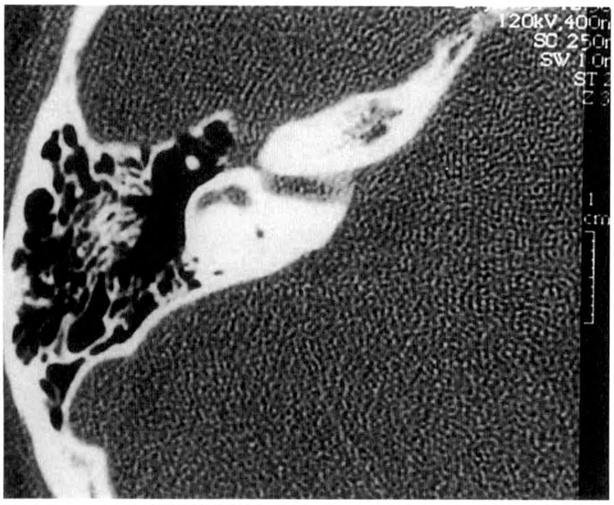


Fig 2. Axial CT scan shows enlargement of geniculate ganglion.

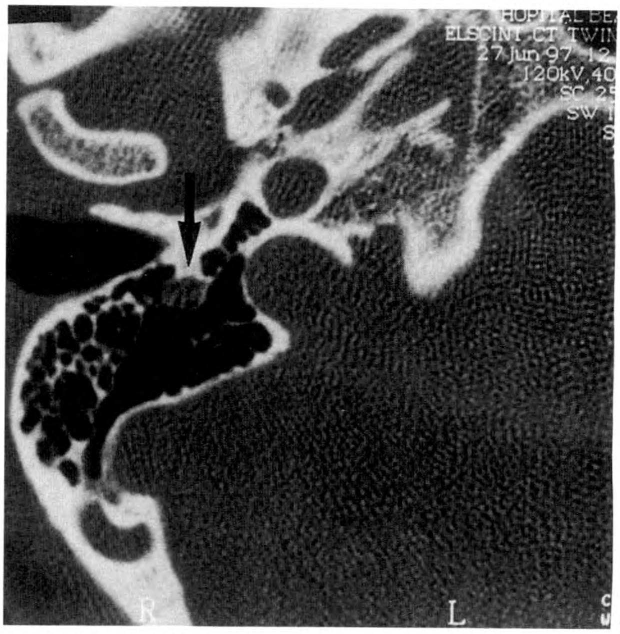


Fig 3. Axial CT scan shows regular enlargement of mastoid segment of facial nerve (arrow).

The facial nerve was totally exposed in the petrous bone from its labyrinthine segment to the trunk of the facial nerve with its main intraparotid branches, through a combined cervical transmastoid, posterior tympanotomy, and middle fossa approach. The labyrinthine and extratemporal segments of the facial nerve appeared normal. The facial nerve was enlarged

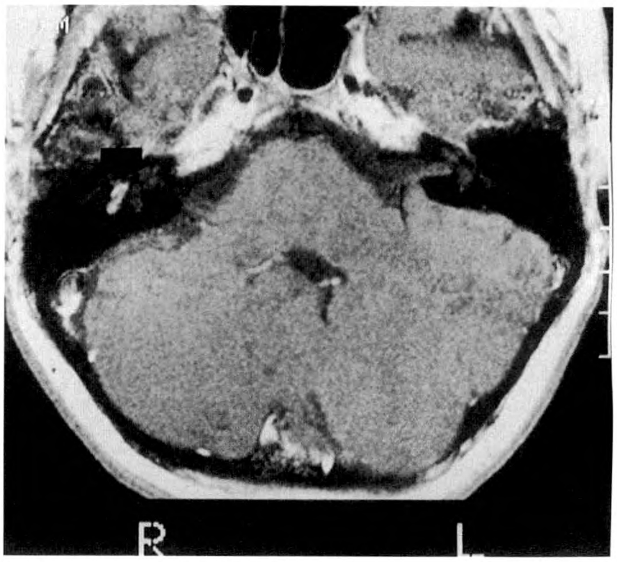


Fig 4. Axial T1-weighted magnetic resonance image made after contrast injection reveals slight and homogeneous gadolinium enhancement of tympanic segment of facial nerve (arrowhead), which looks pseudonodular.

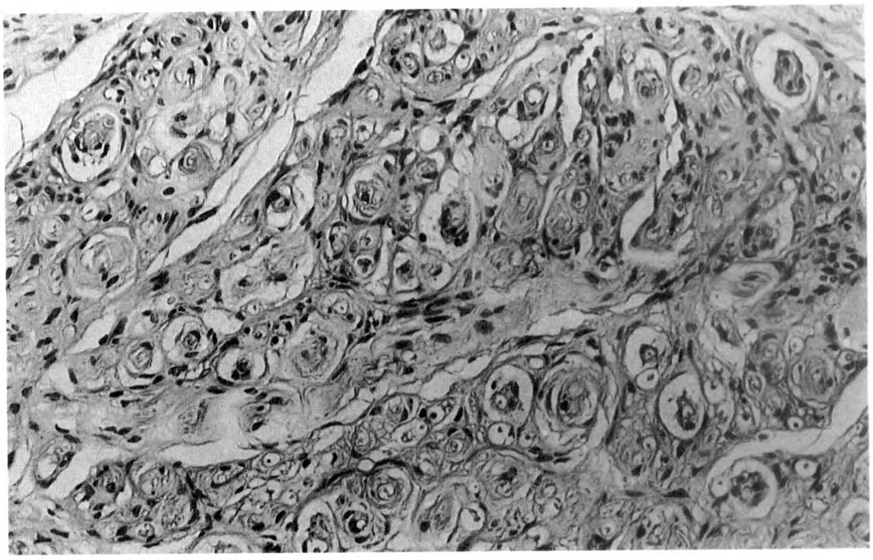


Fig 5. Facial nerve is distended by whorl formation centered by axons creating typical onion bulb formations (H & E, original ×200).

from its labyrinthine segment to the stylomastoid foramen. The proximal and distal stumps were examined during the operation, with the initial suspicion of a tumoral lesion. Pathological examination revealed that both stumps were free of tumor. All of this portion of the intratemporal facial nerve was resected. Removal of the branches of the stapes and the head of the malleus was required. Frozen sections of the proximal and distal tips of the nerve were free of lesion. A 5-cm graft was harvested from the right greater auricular nerve and interposed between the labyrinthine segment and the extratemporal stem of the facial nerve. The proximal nerve graft anastomosis was performed in the auditory internal canal, and the distal one was performed with the extracranial portion of the nerve. The anastomoses were performed with fibrin tissue adhesive (Tissucol, Immuno, Vienna, Austria). Ossiculoplasty was performed with a hydroxyapatite prothesis (total ossicular replacement prosthesis, Xomed, Jacksonville, Fla).

The excised specimen was fixed in 10% buffered formalin, processed for paraffin embedding, and stained with hematoxylin and eosin. Immunohistochemical studies were performed with monoclonal antibodies against S-100 protein (Immunotech Coulter, Miami, Fla), neurofilament subunit (Dako A/S, Glostrup, Denmark), and epithelial membrane antigen (EMA; Dako A/S) according to a 3-step immunoperoxidase method.[11] Macroscopically, the specimen consisted of a 3-cm segment of enlarged and fusiform nerve without defined mass. Histologic analysis of cross sections showed an enlarged hypercellular nerve fascicle without architectural distortion (Fig 5). A diagnosis of schwannoma was easily excluded: central axons were surrounded by an increased number of Schwann cells, creating unusual whorled lesions of various sizes called onion bulb formations. These onion bulb formations reacted with S-100 protein antibody. Neurofilament immunostaining confirmed a normal axon distribution, and an EMA marker did not reveal any perineural cell proliferation (Fig 6). This histologic and immunohistochemical pattern was characteristic of what is described in hypertrophic neuropathy, diffuse or focal. The final histologic examination showed that the pathological process of hypertrophic neuropathy was also present at each end of the nerve specimen. Such a diagnosis requires a complete morphological and immunohistochemical analysis and therefore cannot reasonably be done during an operation.

To rule out a diffuse hypertrophic neuropathy, we performed neurologic and electromyographic examinations of the 4 limbs. The results of the neurologic examination of the 4 limbs were normal, and those of the nerve conduction studies were normal. However, electromyographic examination of the left facial nerve revealed few motor unit potentials in the eye and orbicularis oris muscles. The eye blink reflex showed normal R1 and R2 latencies, with a polyphasic R1 response. These electromyographic findings suggested a subclinical anomaly of the left facial nerve, without any radiologic evidence.

The patient's recovery was uneventful. Clinical examination of the cranial nerves after the operation revealed hypoesthesia within the right V3 area (distribution of the third division of the trigeminal nerve). The pure tone audiogram revealed mixed deafness, with pure tone thresholds ranging from 40 to 80 dB (Rinne test, 0 to 40 dB). The facial nerve grade reached grade III after 1 year of follow-up.

DISCUSSION

Localized hypertrophic neuropathy involves iso-

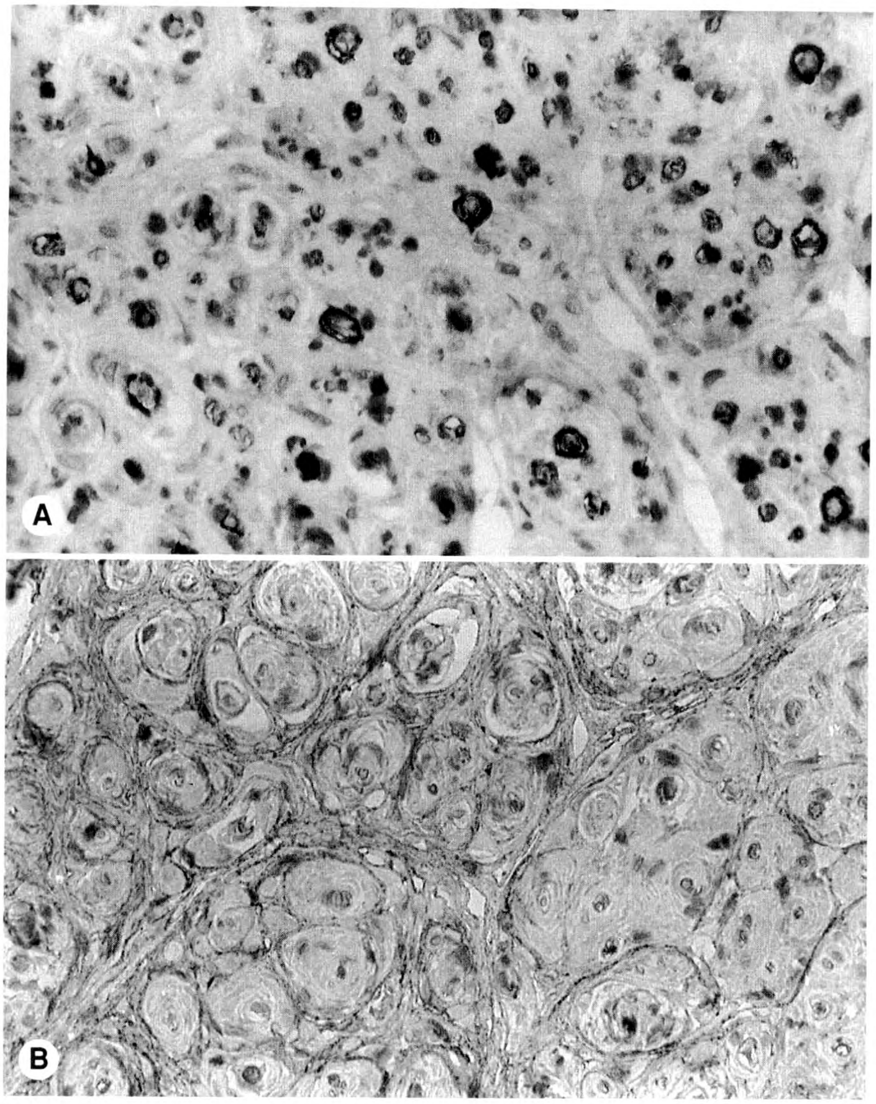


Fig 6. Immunostaining (original ×400). A) Positive immunostaining for S-100 protein in cells composing onion bulbs. B) Negative immunostaining for epithelial membrane antigen in onion bulbs and discrete positivity of some rare perineurial cells around bundles of nerve fibers.

lated nerves. It is a rare entity; only 43 cases of LHN involving peripheral nerves or cranial nerves have been reported in the literature.[4] Cranial nerve involvement by LHN is rare. Cases have been reported in the literature involving the trigeminal nerve (3 cases) and the acoustic nerve (2 cases),[5-9] and 1 case of intratemporal facial nerve perineurioma has been documented[4] (see Table). In this report, we describe the first case of LHN involving the facial nerve with enough well-documented pathological data to exclude the differential diagnostic entities (perineurioma and generalized hypertrophic neuropathies).

LITERATURE REPORTS OF LOCALIZED HYPERTROPHIC NEUROPATHY INVOLVING ISOLATED CRANIAL NERVES

Case	*Authors*	*Year*	*Cranial Nerve*	*Reactivity*
1	Baskin et al[5]	1981	V	NP
2	Chang et al[6]	1993	V	EMA−; S-100+
3	Kusama et al[7]	1981	V_3	NP
4	Neely et al[8]	1981	VIII	NP
5	Brawley and Kelly[9]	1966	VIII	NP
6	Li et al[4]	1996	VII	EMA+; S-100−

NP — not performed. EMA — epithelial membrane antigen.

Perineurioma is considered the main differential diagnosis, because its histologic appearance is similar to that of LHN. Perineurioma is a tumor that originates from well-differentiated perineurial cells without Schwann cell proliferation. The proliferative perineurial cells form onion bulbs around the axon, similar to those seen in LHN, and are responsible for tumoral enlargement of the nerve.[12-16] The immunohistochemical findings are characteristic: perineurial cells in onion bulb formations exhibiting positive reactivity for EMA and negative reactivity for S-100 protein.[4,12-16] In our reported case, onion bulb formations exhibited the reverse pattern: they were positive for S-100 protein and negative for EMA, thus ruling out a perineurial origin. Only 1 case of perineurioma involving cranial nerves has been documented in the literature, and it was also initially considered suspicious for an intratemporal facial schwannoma.[4]

The histologic picture of onion bulb formations is generally seen in diffuse hypertrophic neuropathies, such as Charcot-Marie-Tooth disease (hereditary motor-sensory neuropathy [HMSN] type I), Dejerine-Sottas disease (HMSN type III), and Refsum disease.[1-3] Onion bulb formations characterize chronic inflammatory demyelinating neuropathies and are also pathological findings of diabetic neuropathy and leprosy.[2] In the reported case, there was no diabetes. There was no support for any hereditary component. Neurologic examination of the 4 limbs yielded normal findings, and electromyography of the 4 limbs failed to detect any demyelinating or axonal polyneuropathy. Therefore, these data are considered sufficient to exclude an early stage of one of those generalized hypertrophic neuropathies.

However, clinical, electromyographic, and magnetic resonance imaging follow-up is required, because other cranial nerves might be involved in this pathological process. In our reported case, clinical examination detected hypoesthesia within the right V3 area, and electromyographic examination of the contralateral facial nerve detected minimal abnormalities such as demyelination. Genetic disorders such as gene abnormalities on chromosome 22 have been described and play a role in the pathogenesis of intraneural perineurioma.[12] In HMSNs, gene mutations of chromosome 22 have also been reported. Therefore, a genetic analysis will be performed in our case to exclude definitively a clinically silent HMSN or an atypical neurofibromatosis with a point mutation on chromosome 22.

The cause of LHN is unknown. Various hypotheses have been formulated regarding whether it is a reactive process or a tumoral process.[12,17] Hypotheses concerning a reactive process, such as trauma, local compression, nerve entrapment, inflammation, a focal perineurial barrier defect, and focal disruption of the blood-nerve barrier, have been postulated.[4,18,19] Onion bulb formations may result from a demyelination-remyelination process,[1,2,8] and this hypothesis is supported by experimental demyelination.[3] In our reported case, there was no history of trauma or of other predisposing conditions; no inflammation or vascular changes were observed on pathological examination.

The treatment of such lesions is debatable. The issue is whether the performed surgical removal with facial nerve grafting was the best treatment, or whether corticosteroids would have induced remission of the lesion. In 1 case of cranial nerve involvement in long-standing demyelinating polyneuropathy, treatment with systemic corticosteroids resulted in no change in the neurologic status.[20] In cases of LHN, systemic corticosteroids are occasionally successful in inducing remission.[9,21,22] Simple decompression of the nerve involved by LHN may improve the patient's condition.[22,23] Our patient presented with a 9-month history of facial paralysis staged as grade II. The lesion was managed as any other primary tumor of the facial nerve, mainly because before operation, this lesion was suspected to be a schwannoma, because of the sudden onset of the disease and the fast evolution of the clinical manifestations. The aim of surgical treatment was twofold: removal of the tumor and preservation of the facial nerve. As it happened, the tumor could not be dissected from the facial nerve and required complete surgical removal with cable graft anastomosis. According to several studies,[24-26] this anastomosis technique using fibrin tissue adhesive offers a good restoration of facial function within 1 year.

We describe a case of LHN affecting the facial nerve with clinical, radiologic, and complete pathological data. Such rare lesions may affect a cranial nerve and differ from perineurioma on immunohistochemical analysis. Complete neurologic examination and follow-up is required in such cases for confirmation of the absence of diffuse neuropathies, which is eventually completed with genetic investigations. Therefore, hypertrophic neuropathy must be included in the differential diagnosis of a primary tumor of the facial nerve, especially when onion bulb formations are detected on pathological examination.

REFERENCES

1. Dyck PJ, Chance P, Lebo R, Carnley JA. Hereditary motor and sensory neuropathies. In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poluslo JF, eds. Peripheral neuropathy. Philadelphia, Pa: Saunders, 1993:1102-10.

2. Dyck PJ. Inherited neuronal degeneration and atrophy affecting peripheral motor, sensory and autonomic neurons. In: Dyck PJ, Thomas PK, Lambert E, eds. Peripheral neuropathy. Philadelphia, Pa: Saunders, 1975:825-67.

3. Dyck PJ. Experimental hypertrophic neuropathy. Arch Neurol 1969;21:73-81.

4. Li D, Schauble B, Fisch U. Intratemporal facial perineurioma. Laryngoscope 1996;106:328-33.

5. Baskin DS, Townsend JJ, Wilson CB. Isolated hypertrophic interstitial neuropathy of the trigeminal nerve associated with trigeminal neuralgia. J Neurosurg 1981;55:987-90.

6. Chang Y, Horoupian DS, Jordan J, et al. Localized hypertrophic mononeuropathy of the trigeminal nerve. Arch Pathol Lab Med 1993;117:170-6.

7. Kusama K, Iwamoto A, Mikuni M, et al. A case of central perineurioma (Lazarus and Trombetta) of the mandible. J Nihon

Univ Sch Dent 1981;23:10-7.

8. Neely JG, Armstrong D, Benson J, Neblett C. "Onion bulb" formation associated with a solitary neoplasm of the eighth nerve sheath. Am J Otolaryngol 1981;2:307-13.

9. Brawley BW, Kelly WA. Interstitial hypertrophic neuritis of the eighth cranial nerve. J Neurol Sci 1966;8:225-37.

10. House JW, Brackmann DE. Facial nerve grading system. Otolaryngol Head Neck Surg 1985;93:146-7.

11. Rouse R, Warnke R. Special applications of tissue section immunologic staining in the characterization of monoclonal antibodies and in the study of normal and neoplastic tissues. In: Weir DM, Blackwell CL, Herrenberg LA, eds. Handbook of experimental immunology. Vol 4. Edinburgh, Scotland: Blackwell, 1986;chap 116:1-16.

12. Emory TS, Scheithauer BW, Hirose T, Wood M, Onofrio BM, Jenkins RB. Intraneural perineurioma: a clonal neoplasm associated with abnormalities of chromosome 22. Am J Clin Pathol 1995;103:696-704.

13. Bilbao JM, Khoury NJS, Hudson AR, Briggs SJ. Perineurioma (localized hypertrophic neuropathy). Arch Pathol Lab Med 1984;108:557-60.

14. Ariza A, Bilbao JM, Rosai J. Immunohistochemical detection of epithelial antigen in perineurial cells and perineurioma. Am J Surg Pathol 1988;12:678-83.

15. Theaker JM, Fletcher CDM. Epithelial menbrane antigen expression by the perineurial cell: further studies of peripheral nerve lesions. Histopathology 1989;14:581-92.

16. Tsang WYW, Chan JKC, Chow LTC, Tse CCH. Perineurioma: an uncommon soft tissue neoplasm distinct from localized hypertrophic neuropathy and neurofibroma. Am J Surg Pathol 1992;16:756-63.

17. Mitsumoto H, Wilbourn AJ, Goren H. Perineurioma as the cause of localized hypertrophic neuropathy. Muscle Nerve 1980;3:403-12.

18. Lazarus SS, Trombetta LD. Ultrastructural identification of a benign perineurial cell tumor. Cancer 1978;41:1823-9.

19. Johnson PC, Kline DJ. Localized hypertrophic neuropathy: possible focal perineurial barrier defect. Acta Neuropathol (Berl) 1989;77:514-8.

20. McCann EL, Smith TW, Chad DA, Sargent J. Severe cranial nerve involvement in longstanding demyelinating polyneuropathy: a clinicopathologic correlation. Acta Neuropathol (Berl) 1996;91:309-12.

21. Austin JH. Recurrent polyneuropathies and their corticosteroid treatment; with five-year observations of a placebo-controlled case treated with corticotrophin, cortisone, and prednisone. Brain 1958;81:157-92.

22. Taras J, Melone CP. Hypertrophic neuropathy presenting with ulnar nerve compression: a case report. J Hand Surg [Am] 1995;20:233-4.

23. Böcker DK, Schönberg F, Gullotta F. Localized hypertrophic neuropathy: a rare, clinically almost unknown syndrome. Clin Neuropathol 1984;3:228-30.

24. Siedentop KH, Loewy A. Facial nerve repair with tissue adhesive. Arch Otolaryngol 1979;105:423-6.

25. Sterkers O, Becherel P, Sterkers JM. Réparation du nerf facial par colle de fibrine exclusive: 56 cas. Ann Otolaryngol Chir Cervicofac 1989;106:176-81.

26. Bento RF, Miniti A. Anastomosis of the intratemporal facial nerve using fibrin tissue adhesive. Ear Nose Throat J 1993; 72:663-72.

BILATERAL LARYNGEAL PSEUDOPARALYSIS IN XANTHOMA DISSEMINATUM TREATED BY ENDOSCOPIC LASER MEDIAL ARYTENOIDECTOMY

GIOVANNA CANTARELLA, MD
CESARE BARTOLOMEO NEGLIA, MD
ANGELO VALERIO MARZANO, MD
ANTONIO OTTAVIANI, MD

MILAN, ITALY

Xanthoma disseminatum is a rare non–Langerhans' cell histiocytosis, characterized by papular cutaneous eruption, possible mucosal involvement, and frequent association with vasopressin-sensitive diabetes insipidus. Herein we report a case of xanthoma disseminatum with pharyngolaryngeal involvement. In this patient, mucosal xanthomas involving the arytenoid cartilages and the interarytenoid area resulted in laryngeal stenosis and severe impairment of both cricoarytenoid joints' motility. Endoscopic CO_2 laser medial arytenoidectomy, according to the technique described by Crumley (1993), and vaporization of interarytenoid xanthomas were successfully performed, thus reestablishing bilateral cordal motility and the laryngeal airway. Four years later, a CO_2 laser revision was necessary because of recurrence of xanthomas in the posterior larynx. Two years after the latter operation, the patient has no signs of laryngeal obstruction and has a normal voice quality. This case report suggests that endoscopic medial arytenoidectomy may be successfully used in the treatment of bilateral laryngeal pseudoparalysis secondary to xanthoma disseminatum.

KEY WORDS — laryngeal paralysis, laser medial arytenoidectomy, xanthoma disseminatum.

INTRODUCTION

Xanthoma disseminatum (XD), also known as Montgomery's syndrome, is a rare normolipemic non-Langerhans' cell histiocytoxanthomatosis, associated with a chronic course and characterized by diffuse cutaneous papular eruption, possible mucosal involvement, and frequent association with vasopressin-sensitive diabetes insipidus.[1,2] It is classified among histiocytoses, ie, a heterogeneous group of

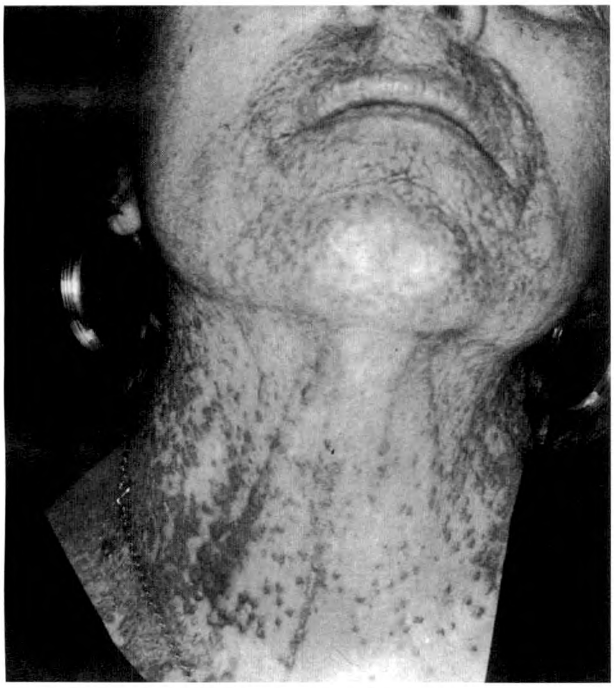


Fig 1. Brownish papular lesions, typical of xanthoma disseminatum, are widely diffused on patient's neck and face.

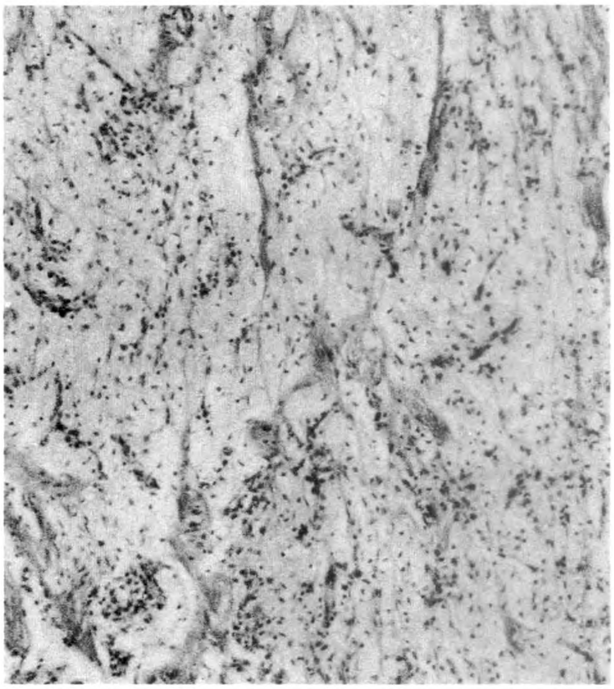


Fig 2. Histologic specimen from cutaneous xanthomatous lesion shows dense dermal infiltrate composed of foamy histiocytes and scattered lymphoid cells (H & E, original ×400).

From the Departments of Otolaryngology (Cantarella, Neglia, Ottaviani) and Dermatology (Marzano), University of Milan, IRCCS Ospedale Maggiore Policlinico, Milan, Italy.

CORRESPONDENCE — Giovanna Cantarella, MD, Dipartimento di Scienze Otorinolaringologiche, Università degli Studi di Milano, IRCCS Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122 Milano, Italy.

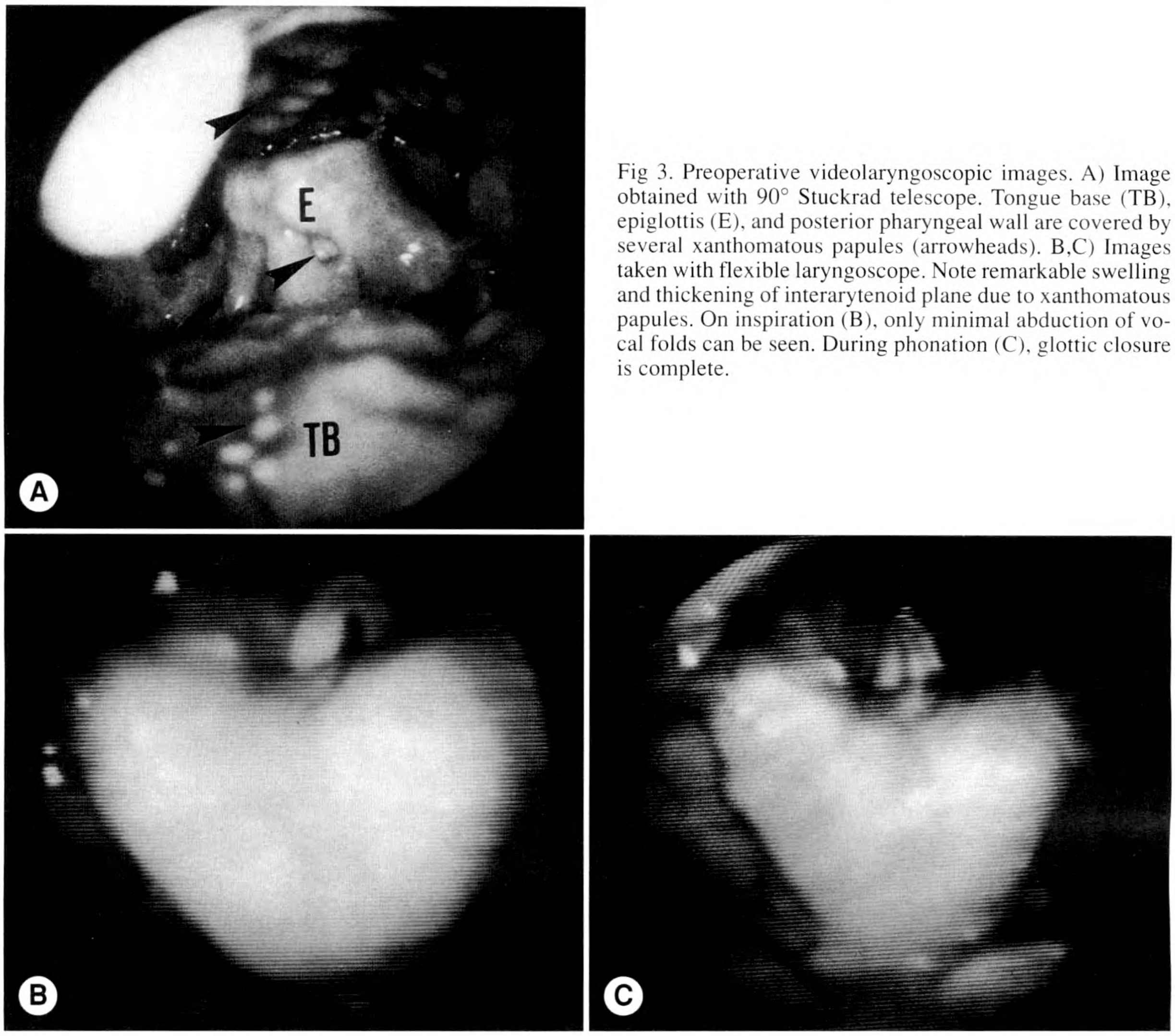


Fig 3. Preoperative videolaryngoscopic images. A) Image obtained with 90° Stuckrad telescope. Tongue base (TB), epiglottis (E), and posterior pharyngeal wall are covered by several xanthomatous papules (arrowheads). B,C) Images taken with flexible laryngoscope. Note remarkable swelling and thickening of interarytenoid plane due to xanthomatous papules. On inspiration (B), only minimal abduction of vocal folds can be seen. During phonation (C), glottic closure is complete.

dysproliferative disorders involving the monocyte-macrophage line.[3] The involvement of the pharyngeal and laryngeal mucosa may cause severe dysphagia and dyspnea. In severe cases of laryngeal obstruction, tracheostomy is required.[4,5]

We describe a case of XD in which extensive laryngeal involvement resulted in airway obstruction and bilateral vocal fold pseudoparalysis. Endoscopic medial arytenoidectomy was performed to widen the posterior glottis.

CASE REPORT

A 36-year-old woman was referred to the department of dermatology of our hospital in September 1991 for a cutaneous eruption involving the face, neck, chest wall, and groin. She also had a 2-year history of vasopressin-sensitive diabetes insipidus.

Her cutaneous lesions were red-brown, itching papules with a yellowish center (Fig 1). The papules were round or oval, with a diameter varying from 2 to 5 mm, with sharp limits. Some of the lesions coalesced to form large brown plaques. Examination of the oral cavity and pharynx showed similar papules on the tongue and soft palate.

The diabetes insipidus was well compensated with vasopressin substitutive therapy. Histologic examination of a cutaneous lesion of the chest wall showed a histiocytic dermal infiltrate with remarkable phenomena of xanthomization (Fig 2). Immunohistochemical stainings for macrophagic CD68 antigen (KIM6 and KP1) were positive on most cells of the infiltrate. Staining for CD1a antigen specific for Langerhans' cells was negative. A diagnosis of XD was made on the basis of the clinical picture and of the histologic and immunohistochemical findings.

The patient underwent esophagogastroduodenoscopy, bone marrow biopsy, skeletal radiographic study, a thorax and abdomen computed tomography (CT) scan, a skull CT scan, and ophthalmologic and endocrinologic evaluations. These examinations did not show any systemic involvement, with the excep-

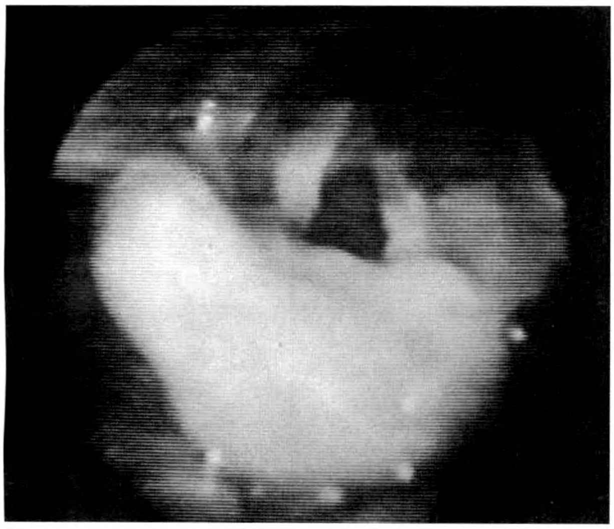


Fig 4. Nasoendoscopic view of larynx during inspiration 10 days after medial arytenoidectomy. Marked improvement of both vocal folds' abduction has been achieved.

tion of diabetes insipidus. Steroid and cytostatic therapy (prednisone, 25 mg daily; cyclophosphamide, 100 mg daily) induced a partial regression of the skin lesions.

One year later, the patient started complaining of effort dyspnea. Three months after the onset of this symptom, she underwent an otolaryngological examination because of worsening dyspnea.

The patient did not complain of dysphagia. The quality of her voice was normal. Pharyngolaryngoscopic examination and fiberoptic endoscopy showed a massive involvement of the oral and pharyngeal mucosa, which was covered by yellow papules coalescing to form plaques (Fig 3A). In the larynx, the spread of the xanthomatous lesions to the arytenoid cartilages and to the interarytenoid space had caused a mechanical blockage of both cricoarytenoid joints, resulting in a severe impairment of inspiratory abduction of the vocal folds (Fig 3B,C). During inspiration, the vocal folds were in a paramedian position; nevertheless, during phonation, a complete glottic closure was attained.

To restore an adequate laryngeal lumen during inspiration, we performed a CO_2 laser medial arytenoidectomy according to the technique described by Crumley.[6] A standard CO_2 laser was used in a pulse mode with a power density of 15 W and a spot size of 0.8 mm. During the arytenoidectomy, care was taken to protect the posterior commissure with saline-soaked cottonoids. The excision was limited to the medial part of the arytenoid cartilage, between the vocal process and the posterior medial corner of the arytenoid cartilage, as recommended by Crumley. The medial arytenoidectomy was carefully completed on the inferior lateral plane, in order to avoid reaching the level of the cricoarytenoid joint. In addition, a vaporization of xanthomas thickening the interarytenoid mucosa was performed on a superficial plane, in order to avoid injury to the underlying interarytenoid muscles.

Immediately after surgery, the patient's dyspnea was relieved. Fiberoptic laryngoscopy revealed a satisfactory reestablishment of the glottic lumen and a nearly complete recovery of the motility of both vocal folds (Fig 4). The quality of the voice was not impaired by the operation.

The patient remained asymptomatic for 4 years. The endoscopic follow-up showed persistence of satisfactory motility of the vocal folds (Fig 5). Forty months after the operation, the patient complained

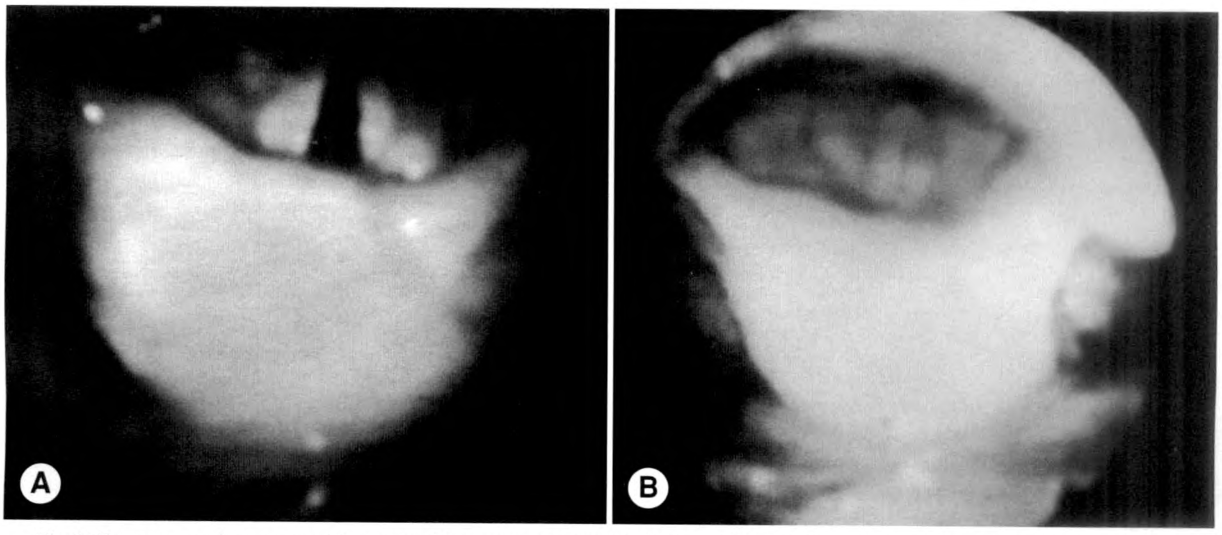


Fig 5. Four years after arytenoidectomy, fiberoptic endoscopy shows mild reduction of glottic lumen on inspiration (A) due to swelling of left arytenoid cartilage and of interarytenoid mucosa. Phonatory adduction of vocal folds is normal (B).

of recurrent dyspnea. At fiberoptic laryngoscopy, new xanthomatous lesions were observed in the interarytenoid area and on the right arytenoid cartilage, opposite to the operated side. Therefore, a CO2 laser revision was performed, and the infiltrated tissues were successfully vaporized, with full recovery of the laryngeal lumen. Two years later, the patient no longer complains of dyspnea, and a recent fiberoptic laryngoscopic examination showed persistence of a normal glottic lumen.

DISCUSSION

Xanthoma disseminatum, first described by Montgomery and Osterberg[7] in 1938, is a histiocytic dysproliferative disease that diffusely involves the skin and may affect the mucous membranes of the aerodigestive tract. Almost 100 cases have been reported so far.[8] Vasopressin-sensitive diabetes insipidus, occurring in about one third to one half of patients, is a marker of systemic involvement, and is due to localization of xanthomatous infiltration at the tuber cinereum, infundibulum hypothalami, and sella turcica.[9]

The diagnosis of XD relies on the clinical picture and on histologic and immunohistochemical findings in cutaneous or mucosal specimens of a dense dermal infiltrate composed of foamy histiocytes and scattered lymphoid cells.[10] Medical therapy hardly influences the disease process.[11,12] The course of the disease is usually benign. The mucocutaneous lesions may persist for life or, sometimes, may resolve spontaneously after several years.[7] Nevertheless, the skin lesions may be disfiguring, and the mucosal involvement may be functionally impairing.[13]

Massive involvement of the pharyngolaryngeal membranes in the course of XD may induce life-threatening dyspnea. In previously reported cases, tracheostomy had to be performed. In a reported series of XD, 6 of 55 patients underwent tracheostomy.[5] As yet, no single study has reported on arytenoidectomy as a treatment for laryngeal stenosis due to XD.

Mechanical blockage of the cricoarytenoid joints secondary to mucosal lesions of the larynx is an infrequent but possible source of glottic stenosis.[14] In our patient, bilateral laryngeal hypomotility occurred because of massive spread of mucosal xanthomas to the arytenoid cartilages and the interarytenoid space. These lesions, although diffusely involving the whole supraglottic larynx, were not the sole cause of the patient's severe laryngeal obstruction. Although the glottis was completely free from lesions, the motility of the vocal folds was impaired, especially during abduction, and the endoscopic picture resembled that of Gerhardt's palsy. Hypomotility of the arytenoid cartilages appeared to be secondary to a mechanical blockage of the cricoarytenoid joints by xanthomatous lesions infiltrating the interarytenoid plane and causing thickening and rigidity of this area. These observations suggested a new therapeutic strategy.

Endoscopic CO2 laser arytenoidectomy is a well-known surgical technique that is widely employed in the treatment of bilateral laryngeal paralysis. In addition, this procedure has been recently proposed for the treatment of pediatric posterior glottic stenosis impairing cricoarytenoid joint motility.[15] In our patient, medial arytenoidectomy was performed according to the technique described by Crumley,[6] and at the same time, the thickened and infiltrated mucosa of the interarytenoid space was vaporized. Unlike total arytenoidectomy, the technique reported by Crumley allows preservation of the vocal and muscular processes and the cricoarytenoid joint, as only the superior and medial portions of the arytenoid cartilage are excised. Therefore, the muscular and ligamentous insertions of the arytenoid cartilage are left intact.

In the reported case, the immediate postoperative result was excellent; the operation not only allowed reestablishment of the breathing space, but also permitted the recovery of motility of the vocal folds soon after surgery. Although, 40 months after the operation, the patient developed a xanthomatous recurrence in the interarytenoid plane, a CO2 laser endoscopic revision reestablished an adequate laryngeal lumen.

Since XD is characterized by a chronic course and a poor response to medical treatment, an indefinite follow-up is necessary. The good functional outcome obtained in this case suggests that medial arytenoidectomy may be an adequate treatment for laryngeal stenosis caused by XD.

REFERENCES

1. Winkelmann RK. Cutaneous syndromes of non X histiocytosis. Arch Dermatol 1981;117:667-72.

2. Altman J, Winkelman RK. Xanthoma disseminatum. Arch Dermatol 1962;86:582-96.

3. Gianotti F, Caputo R. Histiocytic syndromes: a review. J Am Acad Dermatol 1985;13:383-404.

4. Varotti C, Bettoli V, Berti E, Cavicchini S, Caputo R. Xanthoma disseminatum: a case with extensive mucous membrane involvement. J Am Acad Dermatol 1991;25:433-6.

5. Beurey J, Lamaze B, Weber M, Delrous JL, Kremer B, Chaulieu Y. Xanthoma disseminatum (syndrome de Montgomery). Ann Dermatol Venereol 1979;106:353-9.

6. Crumley RL. Endoscopic laser medial arytenoidectomy for airway management in bilateral laryngeal paralysis. Ann Otol

Rhinol Laryngol 1993;102:81-4.

7. Montgomery H, Osterberg AE. Xanthomatosis: correlation of clinical, histopathologic, and chemical studies of cutaneous xanthoma. Arch Dermatol Syphilol 1938;37:373-402.

8. Caputo R, Veraldi S, Grimalt R, et al. The various clinical patterns of xanthoma disseminatum. Dermatology 1995;190: 19-24.

9. Mamou H, Carteaud A, Lumbruso A. Diabete insipide et xanthomes disseminés. Semin Hop 1953;26:1311-5.

10. Zelger BWH, Sidoroff A, Orchard G, Cerio R. Non Langerhans cell histiocytoses. Am J Dermatopathol 1996;18:490-504.

11. Szekeres E, Tiba A, Korom I. Xanthoma disseminatum: a rare condition with non X non lipid cutaneous histiocytopathy. J Dermatol Surg Oncol 1988;14:1021-4.

12. Kuligowski M, Gorkievicz-Petkow A, Jablonska S. Xanthoma disseminatum. Int J Dermatol 1992;31:281-3.

13. Bromley GS, Grulian D. Xanthoma disseminatum: an unusual cause of facial and limb deformity. Plast Reconstr Surg 1983;72:552-6.

14. Bogdasarian RS, Olson NR. Posterior glottic laryngeal stenosis. Otolaryngol Head Neck Surg 1980;88:765-72.

15. Rimell FL, Dohar JE. Endoscopic management of pediatric posterior glottic stenosis. Ann Otol Rhinol Laryngol 1998; 107:285-90.

≈

MANAGEMENT OF THE TINNITUS PATIENT

The Ninth Annual Conference on the Management of the Tinnitus Patient, for professionals and tinnitus patients, will be held in Iowa City, Iowa, October 4-6, 2001. For information, see web site www.medicine.uiowa.edu/otolaryngology/news/news or contact Rich Tyler by telephone (319-356-2471) or e-mail (rich-tyler@uiowa.edu).

Ann Otol Rhinol Laryngol 110:2001

TRACHEAL LENGTH OF INFANTS UNDER THREE MONTHS OLD

KUO-SHENG LEE, MD CHENG-CHIEN YANG, MD

TAIPEI, TAIWAN

The measurement of tracheal length in infants is difficult to perform in vivo. In adults, tracheal length may be consistent with age, but in infants, tracheal length may vary much more with age and other factors. This study used video rigid ventilation bronchoscopy to evaluate the length of the airway, concentrating on the population younger than 3 months old. There were 34 infants in this study: 14 boys and 20 girls. The mean length from the superior border of the vocal fold to the carina was 5.04 cm, and the mean tracheal length (from the ridge of the first tracheal ring to the carina) was 4.12 cm. There was no significant difference between boys and girls in the length from the vocal fold to the carina or in the tracheal length. The length from the vocal fold to the carina is best correlated to body weight, followed by body height and age.

KEY WORDS — infant, tracheal length, video ventilation bronchoscopy.

INTRODUCTION

There have been many reports on tracheal length in adults. However, there are not many reports on that of infants. The reason is that tracheal length measurement in infants is difficult to perform in vivo. The few such reports on infants are gathered from autopsy, radiologic study, and endoscopy.[1-5] However, autopsy is limited in revealing the living situation. Radiologic study gives accurate results on the diameter of the trachea and the angle of the carina, but it cannot produce the precise length of the trachea being observed. Such precision cannot be achieved because the infants' condition and posture can hamper the assessment and thereby affect the results. Thus, endoscopic examination is a more natural way of measuring the tracheal length of infants, even though it would extend the patient's neck.[1-3,6,7]

The tracheal length of an infant changes rapidly as he or she ages. Consequently, for a study, infants should be categorized into distinct age groups to yield more accurate results.

Our study measured the length of the respiratory tract of infants younger than 90 days old under video ventilation bronchoscopy. The distance from the vocal fold to the carina was statistically analyzed in relation to gender, age, body weight, and body height.

MATERIALS AND METHODS

Thirty-four infants younger than 90 days old were examined by the same two physicians throughout the process and underwent video rigid ventilation bronchoscopy for either examination or therapy from January 1997 to December 1998 at Mackay Memorial Hospital in Taiwan. Infants with congenital subglottic stenosis or congenital tracheal anomalies were excluded from the study. The tracheal length was measured from the ridge of the first tracheal ring to the carina. The length from the vocal fold to the carina was measured from the superior border of the vocal fold to the carina.

With the patient under general anesthesia, a Karl Storz rigid ventilation bronchoscope was applied and connected to a video system. During the measuring process, ventilation was discontinued. The anatomic landmarks of the airway and the tip of the scope were clearly projected in a magnified picture on the monitor, so that the distances from the upper incisor to the carina, the upper incisor to the ridge of the first tracheal ring, and the upper incisor to the superior border of the vocal fold could be measured. As a result, the length of the trachea and the length from the vocal fold to the carina could be calculated. The length from the vocal fold to the carina was analyzed in relation to gender, age, body weight, and body height. Finally, Student's *t*-test and a simple regression analysis were used to reveal the statistical significance of these variables.

RESULTS

Thirty-four infants (14 boys and 20 girls) made up the sample for this study (Table 1). Four were born prematurely, and the rest were full-term. The diagnoses of the 34 young patients made by ventilation bronchoscopy included laryngomalacia, unilateral or bilateral vocal palsy, subglottic edema, and lung atelectasis (Table 2). These infants had a mean age of 46 days, a mean body weight of 3,503.4 g, a mean

---

From the Department of Otolaryngology–Head and Neck Surgery, Mackay Memorial Hospital, Taipei, Taiwan.

CORRESPONDENCE — Kuo-Sheng Lee, MD, Dept of Otolaryngology–Head and Neck Surgery, Mackay Memorial Hospital, 92, Chung-Shan North Road SEC.2, Taipei 10449, Taiwan.

TABLE 1. AGE DISTRIBUTION OF PATIENTS (N = 34)

Age (d)	*Male*	*Female*
1-30	4	8
31-60	3	7
61-90	7	5
Total	14	20

body height of 52.71 cm, a mean length from the vocal fold to the carina of 5.04 cm, and a mean tracheal length of 4.12 cm. There was no significant difference between the sexes in age, body weight, body height, length from the vocal fold to the carina, or tracheal length (Table 3).

In a simple regression analysis comparing the length from the vocal fold to the carina with age, body weight, and body height, the length from the vocal fold to the carina was best correlated to body weight. There was also a significant correlation to body height and age (Table 4). In the male patients, the length from the vocal fold to the carina was best correlated to body weight, followed by body height. However, there was no significant correlation to age in our study (Table 5). In the female patients, the length from the vocal fold to the carina was most significantly correlated to body weight, followed by body height and age (Table 6).

DISCUSSION

In general, the respiratory tract of infants is short. A more precise measurement obtained for this population would be very valuable during tracheal surgery, endotracheal tube insertion, head and neck surgery, and physiological studies.[3,4,8] The tracheal length is rather fixed for teenagers, whereas it varies with age for infants.[3,9] Therefore, in this study, the tracheal lengths of patients younger than 3 months old were measured to obtain more precise data for this population. At the same time, relevant variables were considered when evaluating the change in the tracheal length of infants.

Fearon and Whalen[1] and Morgan and Steward[8] had also studied the tracheal length via rigid ventilation bronchoscopy under general anesthesia. Even though neck extension cannot be prevented during this process, the method of measurement yields the results closest to the in vivo condition. However, identification of the cricoid cartilage is more difficult via radiologic study or direct visualization through a rigid ventilation bronchoscope than it is with video. Our study process was somewhat similar to those of the other studies, except that the bronchoscope was connected to a video system for better evaluation. With the image projected and magnified on the monitor, the anatomic locations of the subglottic area, cricoid cartilage, and ridge of the first tracheal ring could be identified more accurately.[10] As a result, a more precise tracheal length measurement was acquired.

TABLE 2. MAJOR DIAGNOSES OF PATIENTS (N = 34)

Major Diagnosis	*No.*
Laryngomalacia	19
Tongue base cyst	5
Vocal palsy (bilateral or unilateral)	6
Lung atelectasis	4
Subglottic edema	5
Congenital nasal pyriform aperture stenosis	1

TABLE 3. MEAN VALUES OF VARIABLES BY GENDER

	Male	*Female*	*p*
N	14	20	
Age (d)	52.57 ± 28.89	41.95 ± 25.45	.60
Height (cm)	53.29 ± 3.02	52.30 ± 3.08	.97
Weight (g)	3,606.71 ± 618.68	3,431.15 ± 696.16	.67
VOCA (cm)	5.14 ± 0.52	4.97 ± 0.59	.63
TRA (cm)	4.18 ± 0.46	4.08 ± 0.60	.32

Differences in values (mean ± SD) are not significant at $p > .05$.

VOCA — length from superior border of vocal fold to carina, TRA — tracheal length.

Using computed tomography, Griscom and Wohl[9] concluded that there was no significant difference in the tracheal length between male and female subjects under 14 years old or between male and female subjects 20 years old. Consistent with that report, the results of our study showed that there was no significant difference between male and female patients in tracheal length or in the length from the vocal fold to the carina.

In this study, the mean length from the vocal fold to the carina was 5.04 cm, and the mean tracheal length was 4.12 cm. These measurements are shorter than those found by Fearon and Whalen[1] and by Morgan and Steward,[8] who found lengths from the vocal fold to the carina of 5.7 cm and 5.9 cm, respectively, in infants under 3 months old. However, the tracheal length in our study is longer than that of Engel's[11] autopsy report (4 cm). Ethnic differences, sample numbers, concurrent diseases, and varying approaches may explain this discrepancy.

Tracheal length constantly changes until the teen-

TABLE 4. REGRESSION ANALYSIS OF LENGTH FROM VOCAL FOLD TO CARINA IN BOTH SEXES (N = 34)

	Coefficient			
Variable	*Intercept*	*Slope*	*r*	*p*
Age	4.667	0.008	.39	.024
Height	0.079	0.094	.51	<.001
Weight	3.088	0.0005	.66	<.001

Values are significantly correlated at $p < .05$.

TABLE 5. REGRESSION ANALYSIS OF LENGTH FROM VOCAL FOLD TO CARINA IN BOYS (N = 14)

Variable	Coefficient		r	p
	Intercept	Slope		
Age	4.871	0.005	.29	.320
Height	–0.026	0.097	.57	<.035
Weight	2.887	0.0006	.75	<.002

Values except for age are significantly correlated at $p < .05$.

TABLE 6. REGRESSION ANALYSIS OF LENGTH FROM VOCAL FOLD TO CARINA IN GIRLS (N = 20)

Variable	Coefficient		r	p
	Intercept	Slope		
Age	4.550	0.010	.43	.049
Height	0.332	0.089	.46	.041
Weight	3.224	0.0005	.60	.005

Values are significantly correlated at $p < .05$.

age years. This study shows that the length from the vocal fold to the carina is statistically correlated to age, body height, and, especially, body weight. However, in male patients, it was not significantly correlated to age. There are more factors related to tracheal length in this age group, but our study shows that body weight, body height, and age may be considered important.

This study reveals that the length from the vocal fold to the carina is most correlated to body weight, with an r value of .66. However, this correlation is lower than the r value of .908 reported by Morgan and Steward,[8] who used a rigid bronchoscope. It is also lower than the r value of .88 of Griscom and Wohl,[9] who used computed tomography. The difference may be due to the specific age group, the number of patients, and the coexisting disease. This study consisted of patients younger than 90 days old, while the other two reports had patient ages ranging from 9 days to 20 years old, with only a few patients under 3 months old.

Ventilation bronchoscopy is rarely performed in normal infants; the patients in our study had one or more disorders. We excluded patients with congenital subglottic or tracheal anomalies in order to reduce the difference in tracheal length from that of normal infants. We applied a rigid bronchoscope to a video system to evaluate the anatomic landmarks. We concentrated on the age group under 90 days old, and the study shows that body weight, body height, and age are important factors in the airway length of infants.

REFERENCES

1. Fearon B, Whalen JS. Tracheal dimensions in the living infant. (Preliminary report.) Ann Otol Rhinol Laryngol 1967;76: 964-74.
2. Coldiron JS. Estimation of nasotracheal tube length in neonates. Pediatrics 1968;41:823-8.
3. Butz RO. Length and cross-section growth patterns in the human trachea. Pediatrics 1968;42:336-41.
4. Loew A, Thibeault DW. A new and safe method to control the depth of endotracheal intubation in neonates. Pediatrics 1974;54:506-8.
5. Bourbeau J, Delfino R, Ernst P. Tracheal size is a determinant of the bronchoconstrictive response to inhaled methacholine. Eur Respir J 1993;6:991-5.
6. Pagtakhan RD, Bjelland JC, Landau LI, et al. Sex difference in growth patterns of the airway and lung parenchyma in children. J Appl Physiol 1984;56:1204-10.
7. Griscom NT, Wohl MEB. Dimensions of the growing trachea related to age and gender. AJR Am J Roentgenol 1986;146: 233-7.
8. Morgan GAR, Steward DJ. Linear airway dimensions in children: including those with cleft palate. Can Anaesth Soc J 1982;29:1-8.
9. Griscom NT, Wohl MEB. Dimensions of the growing trachea related to body height. Am Rev Respir Dis 1985;131:840-4.
10. Holinger LD, Green CG. Anatomy. In: Holinger LD, Lusk RP, Green CG, eds. Pediatric laryngology and bronchoesophagology. Philadelphia, Pa: Lippincott-Raven, 1997:19-27.
11. Engel S. The child's lung. Developmental anatomy, physiology and pathology. London, England: Arnold, 1947.

Ann Otol Rhinol Laryngol 110:2001

LONG-TERM CHANGES IN OFF-LESION ENDOCOCHLEAR POTENTIAL AFTER INDUCTION OF LOCALIZED LESIONS IN THE LATERAL WALL

RONG WU, MD, PHD
TOMOYUKI HOSHINO, MD, PHD
HAMAMATSU, JAPAN

Localized lesions were produced in various turns of the guinea pig cochlea by means of a photochemical reaction between systemically administered rose bengal dye and green light illumination. The endocochlear potential (EP) was measured at various off-lesion sites, and a morphological examination was performed. In a previous study, this same investigation was done at 3 days, at which time all sites apical to the lesion showed significant EP depression, and damage to the stria vascularis at the lesion was ongoing. In the present 2-week study, the apical EP values were not different from the basal values, and all experimental values were essentially the same as the EP values found in control animals. Morphological examination revealed that the previously damaged structures were greatly repaired. Localized damage and early apical EP depression followed by damage repair and eventual EP recovery could account for the clinical course of certain cases of idiopathic sudden hearing loss involving low-tone deafness.

KEY WORDS — cochlea, guinea pig, photochemical reaction, rose bengal, sudden hearing loss, thrombus formation.

INTRODUCTION

A circulation disorder of the cochlea has often been quoted as being one of the most probable causes of idiopathic sudden hearing loss, but that claim is not unanimously accepted because of a scarcity of evidence, either in humans or in animals. Successful animal models have not been constructed to explain the clinical picture of the disease,[1-3] the characteristics of which include various types of hearing loss and the reversibility of such loss.

A photochemical reaction has been successfully adopted to produce thrombus formation in vessels of the cerebrum, retina, and inner ear.[4-6] Lesions can be made at any portion of the organ, wherever the light touches. A number of photosensitizing dyes produce various reactive oxygen species when illuminated. One such dye, rose bengal (RB), has been found to be one of the most efficient photodynamic generators when illuminated with green light. The photochemical reaction produces singlet molecular oxygen and oxygen radicals, which subsequently react with structural proteins and lipids in the endothelial cell membrane and initiate direct peroxidation reactions, stimulating the adhesion and aggregation of platelets in the microvessels.[7,8]

Using RB dye and focused green light, we have successfully produced small lesions in the lateral wall of the guinea pig cochlea.[9-13] In a previous paper, we reported that such lesions suppress the endocochlear potential (EP) at distant points apical to the lesion, but have no effect on the EP basal to the lesion. This effect was not apparent during the first 30 minutes after illumination, but it was observed at 3 days.[12]

This article reports the long-term effects of photochemically induced lesions on the EP measured at off-lesion sites in the guinea pig cochlea.

MATERIALS AND METHODS

Animal Preparation. Twenty-five female albino guinea pigs (48 ears), weighing 250 to 350 g, with a normal Preyer's reflex, were used in the present study. The animals were anesthetized with an intraperitoneal injection of 35 mg/kg of pentobarbital sodium. A catheter was inserted into the jugular vein for infusion of RB dye (Wako, Osaka, Japan). The animals' body temperatures were maintained at 37.0°C to 38.0°C throughout the experiment with the aid of a heating pad. The tympanic bulla was exposed by the ventrolateral approach, and a small opening was made. Lesions were produced by means of a photochemical reaction following the procedure described below. The wounds were closed, and the animals were returned to their cages to await testing and investigation.

The animal care and experimental procedures used were approved by the Animal Welfare Committee of the Hamamatsu University School of Medicine.

From the Department of Otolaryngology, Hamamatsu University School of Medicine, Hamamatsu, Japan. This work was supported by the Acute Profound Deafness Research Committee of the Ministry of Health and Welfare, Japan, and the Society for Promotion of International Otorhinolaryngology.

CORRESPONDENCE — Rong Wu, MD, PhD, Dept of Otolaryngology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-3192, Japan.

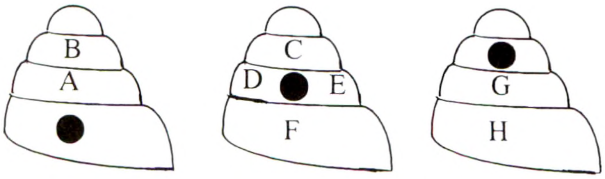


Fig 1. Lesion and endocochlear potential (EP) measurement sites. Illustrations represent guinea pig cochleae (left ear), with basal turn at bottom and apical turn at top. Circles — lesion sites; A-H — EP measurement sites.

Procedure for Photochemical Reaction. Rose bengal dye was dissolved in physiological saline solution (20 mg/mL) and injected (20 mg/kg) over a period of 10 seconds through the catheter placed in the jugular vein. Simultaneously with the injection, the first turn (12 ears), second turn (20 ears), or third turn (11 ears; total, 43 ears) of the cochlea was perpendicularly illuminated for 10 minutes with 1-mm-diameter focused green light from a 75-W xenon lamp (L4887, Hamamatsu Photonics, Hamamatsu, Japan). The wavelength was a 54-nm-wide band centered at 540 nm. After passing through a heat-absorbing green filter, the light was conducted by means of an optic fiber, the head of which was fixed about 15 mm from the surface of the cochlear wall. To avoid any influence from scattered light, the light was directed through a 1-mm-diameter hole in a sheet of black paper covering the bony cochlear wall. The intensity of the green light on the lateral wall was about 8,700 lux.

The cochlear walls in 5 control animals were illuminated with the same green light but without infusion of RB. Only the first turn was illuminated, with the EP measured at the second turn.

Electrophysiological Testing. At 2 weeks all animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (35 mg/kg), and a tracheotomy was performed for an artificial respirator (New England Medical Instruments, Medway, Mass). The wounds were reopened in all animals. The illuminated sites were easily recognizable, because they were covered with mucous membrane with a hypovascular or avascular spot at the point of illumination.

The bony cochlear wall covering the spiral ligament was carefully eroded with a small elevator until the spiral ligament was exposed, whereupon a 50- to 100-µm fenestration was made with a sharp steel needle. A micropipette (tip diameter, 2 to 3 µm) containing 150-mmol/L potassium chloride, with an impedance of approximately 25 MΩ, was placed on the outer surface for the measurement of the EP. After compensating for junction potential, we slowly advanced the micropipette through the lateral wall tissues into the scala media until a positive endolymphatic resting potential was observed. A chloridized silver wire was inserted into the exposed neck muscle to serve as the reference electrode.

The EP measurements were performed at various off-lesion sites as shown in Fig 1. The drawings depict the 4 turns of the cochlea (left ear). Solid circles mark the illuminated site or lesion, the letters A-C and F-H indicate measurement sites in different cochlear turns, and D and E indicate measurement sites 1 mm apical (D) and 1 mm basal (E) to a second-turn lesion. Only 1 EP measurement was performed per ear.

After constant EP values were attained, the respirators were stopped for 2 minutes to induce anoxia and ascertain that the decline in EP caused by anoxia was reversible.

Morphological Study. After electrophysiological data had been collected, the cochleae were removed and perfused with 2.5% glutaraldehyde in 0.1-mol/L phosphate buffer via the round and oval windows, and then immersed in the same fixative for 2 hours.

Three cochleae were decalcified with 5% trichloroacetic acid, dehydrated in graded ethanol, and embedded in celloidin. The cochleae were cut parallel to the modiolus at 20 µm, and each 10th section was stained with hematoxylin and eosin and mounted on a glass slide for light microscopy.

The remaining cochleae were further dissected out under a stereomicroscope, and the surface specimen technique was used for examination by scanning electron microscope. The specimens were treated in a 2% tannic acid solution for 1 hour and then postfixed in 1% buffered osmium tetroxide solution for 1 hour, dehydrated through a graded ethanol series, immersed in methyl propanol, freeze-dried in a freeze-drying apparatus (JEOL, JFD-300, Tokyo, Japan), coated with gold in a sputter coater (JEOL, JFC-1100), and examined with a scanning electron microscope (Hitachi S-800, Tokyo). The locations of insertion holes for EP measurement, together with lesions of the stria vascularis and the organ of Corti, were documented.

Statistical Analysis. A 1-factor analysis of variance was performed with Statview-J 4.5 (Abacus Concepts, Inc, Berkeley, Calif), with a p level of <.01 accepted as statistically significant. The results were expressed as mean ± SEM.

RESULTS

Electrophysiological Data. The EP value of the second cochlear turn in the control animals was found

OFF-LESION EP VALUES AT 2 WEEKS AFTER PHOTOCHEMICAL REACTION

Lesion Site	*Measurement Site*	*n*	*Mean EP ± SEM (mV)*
Basal turn	A	6	74.8 ± 3.2
	B	6	80.7 ± 1.3
Second turn	C	5	74.8 ± 1.3
	D	5	78.0 ± 1.9
	E	3	76.7 ± 0.9
	F	7	79.6 ± 3.3
Third turn	G	6	80.3 ± 1.6
	H	5	81.8 ± 1.1

A-D indicate endocochlear potential (EP) measurements apical to lesion; E-H indicate EP measurements basal to lesion.

to be 81.3 ± 4.3 mV (n = 5), which is not significantly different from the normal control value of 81.4 ± 0.8 mV observed in our previous study ($p > .01$).[13]

Two-Week Off-Lesion EP Measurements. The EP values at the various off-lesion sites are shown in the Table (n = 43). The apical values (A-D) and basal values (E-H) were not significantly different from each other or from the control values ($p > .01$).

Effects of Anoxia on EP. The validity of each measurement was evaluated by observation of the EP during the transient anoxia produced by stopping the respirator. By 2 minutes, the EP had decreased to a negative value (−3.5 ± 1.7 mV). After we restarted the respirator, the recovery of the EP was rapid during the first minute, with a slight overshoot at 4 to 5 minutes before leveling off to the pretest value. The effect of anoxia was the same in both experimental and control animals at 2 weeks.

Morphological Study. The illuminated portion of the second turn showed a marked decrease in cell population under the light microscope (Fig 2B). The stria vascularis had disappeared, and its endolymphatic surface was covered with a thin layer of flat cells. Although the spiral ligament, the organ of Corti, and the spiral limbus retained their original shapes, a marked decrease in fibrocytes, outer and inner hair cells, and interdental cells was observed. The number of spiral ganglion cells in Rosenthal's canal was also markedly decreased. Reissner's membrane seemed to be in its normal position, and the tectorial membrane looked normal (Fig 2B). For comparison, the lesion of a 3-day animal (second turn) from our previous study is shown in Fig 2A. Disintegration of the stria vascularis and a slight decrease in fibrocytes near the inner sulcus are seen.

A scanning electron micrograph from a 2-week cochlea (second turn) shows a scarlike strial lesion covered with irregular flat cells (Fig 3B). Hair cell degeneration was repaired by surrounding supporting cells on the organ of Corti facing the lesion (Fig 4B). The insertion hole for the glass electrode used for EP measurement was clearly seen in the middle of a normal-appearing stria vascularis (Fig 3B).

For comparison, the lesion of a 3-day animal obtained from our previous study is shown in Figs 3A and 4A. Swelling and rupture of the marginal cells

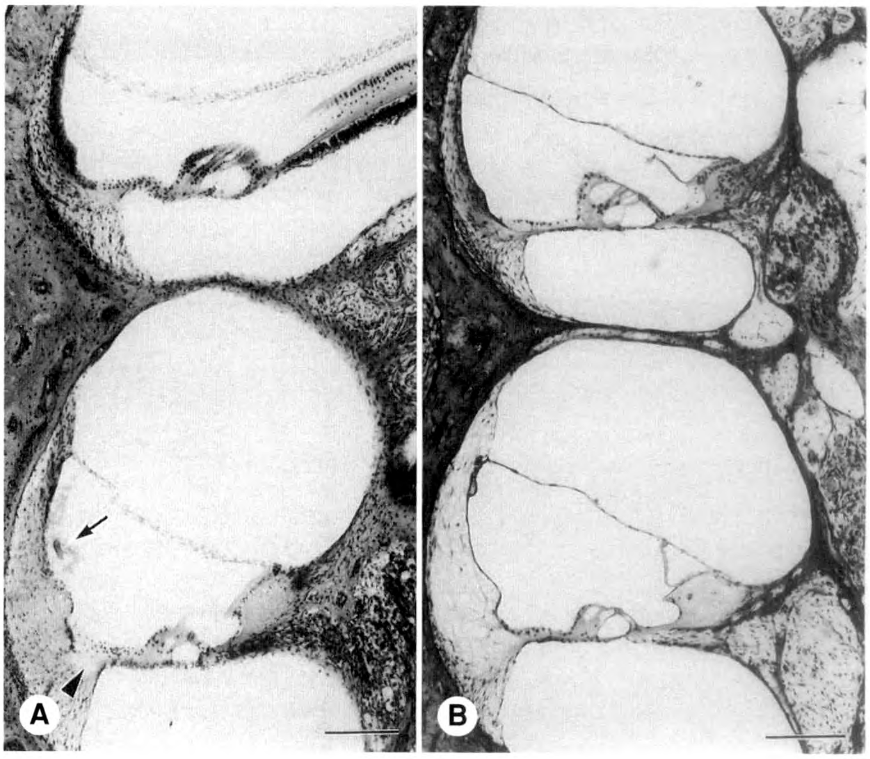


Fig 2. Light microphotographs of midmodiolar sections. Scale bars — 250 μm. A) At 3 days, disintegration of illuminated portion of stria vascularis (arrow) is seen in second cochlear turn, with slight decrease of fibrocyte near inner sulcus (arrowhead). No abnormal changes are seen in nonilluminated upper turn. B) At 2 weeks, illuminated second cochlear turn shows marked decrease in cell population. Stria vascularis has disappeared, and endolymphatic surface is covered with layer of flat cells. Marked decreases in fibrocytes in spiral ligament and limbus spiralis, as well as disappearance of sensory hair cells and spiral ganglion cells, are observed. Interdental cells of spiral limbus have also disappeared. No abnormal changes are seen in nonilluminated upper turn. Neither endolymphatic hydrops nor collapse is seen.

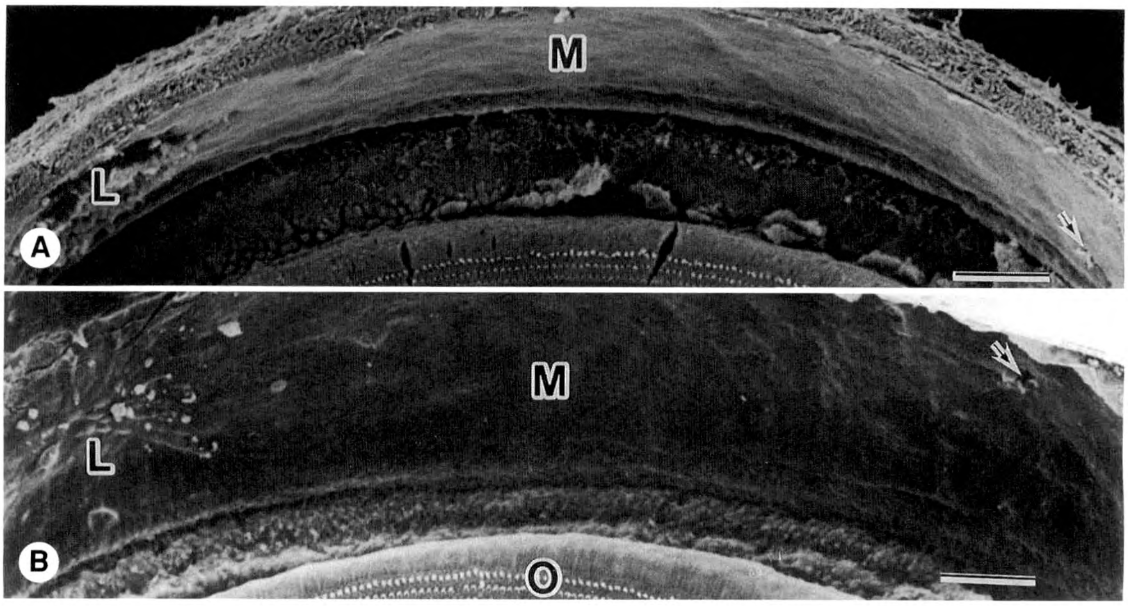


Fig 3. Scanning electron micrographs of lateral walls. Scale bars — 100 μm. A) At 3 days, a spot of disintegration is seen in stria vascularis in second turn (L). Region of normal-appearing marginal cells (M) is found between lesion and apical-located electrode insertion hole for EP measurement (arrow). B) At 2 weeks, second-turn focal lesion is seen to be repaired by larger flat cells (L). Marginal cells (M) and outer hair cells (O) appear normal between lesion and apical EP measurement site (arrow).

are prominent at the illuminated site in the stria vascularis (Fig 3A). Ongoing sensory hair cell changes are also seen facing the degenerating stria vascularis (Fig 4A). The insertion hole for EP measurement (Fig 3A) is seen 1 mm apical to the lesion, but the adjoining areas of the stria vascularis and the organ of Corti look normal. The control ears showed no change at the illuminated portion of the stria vascularis under the scanning electron microscope.

DISCUSSION

In the present study we found that a depressed EP at sites apical to the lesion recovers by 2 weeks after lesion production, and this recovery accompanies a healing of the damaged tissue of the lesion. To our knowledge, similar findings have not been reported in other studies.

In contrast to surgical methods, such as those used by Hallén et al,[14,15] a carefully conducted photochemical reaction can produce circulation disorders at any intended site without surgical damage or heat effects. In a series of studies,[9,10,12,13] we refined our technique so that we were able to produce segmental lesions of about 0.9 mm in any turn of the guinea pig cochlea by focusing green light perpendicular to the cochlear bony wall.

In one of our previous studies,[11] thrombus was observed in the strial capillaries 5 minutes after illumination, and marginal cell degeneration was clearly seen at 90 minutes under a transmission electron microscope. Degeneration of the stria vascularis and organ of Corti reached a maximum 3 days after the photochemical reaction. After 1 week, damage to the

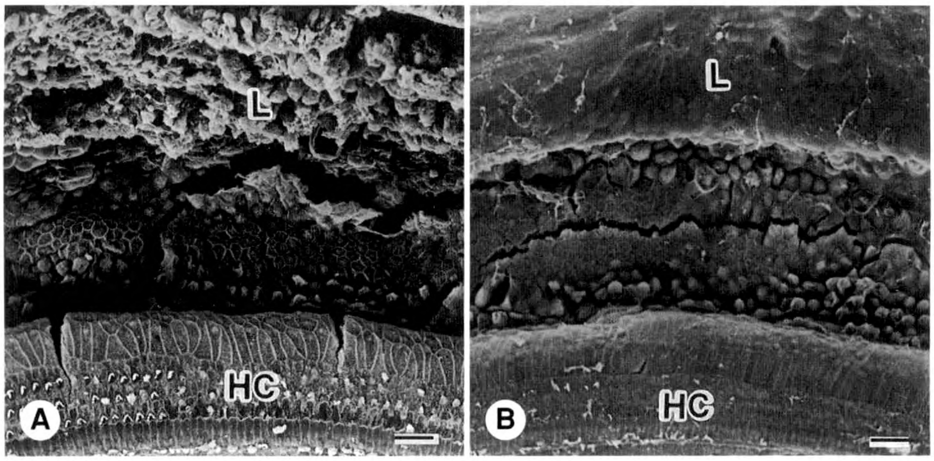


Fig 4. Scanning electron micrographs of photochemically induced lesions. Scale bars — 25 μm. A) At 3 days, swelling and rupture of marginal cells (L) are seen in illuminated portion of stria vascularis. Sensory hair cells facing strial lesion (HC) show ongoing degeneration. B) At 2 weeks, lesion (L) has repaired into irregular epithelial scar. Degenerated hair cells (HC) have been replaced by surrounding supporting cells.

stria vascularis was found to be repaired by a layer of flat cells, and the organ of Corti had also been repaired by enlarged supporting cells.

In another study,[13] the EP measured at midlesion was seen to follow a course similar to that observed for morphological changes. The EP was gradually depressed soon after illumination, reached its lowest value at 3 days, and showed slight recovery at 1 week. At 2 weeks, the midlesion EP values were not significantly different from the values seen in control animals.

From the above, we see that the initial depression and subsequent recovery of the EP corresponds to — and perhaps results from — the initial damage and subsequent repair of the endolymphatic boundary tissue. A survey of the literature shows other studies of the cochlea with findings that follow this same pattern. Poje et al[16] observed a transient decrease in the EP in chicks after exposure to a loud sound, and proposed that structural repair in the tegmentum vasculosum reestablished the potassium concentration in the scala media, as well as the ionic barrier across the papilla. Melichar et al[17] reported reversible changes in the EP and the potassium ion concentration in guinea pigs after acoustic trauma. The EP was measured at the stimulated locus. They attributed the EP depression to pathological changes in the stria vascularis that were characterized by vasoconstriction, and attributed the subsequent return to pretrauma levels to permeability changes in the cochlear partitions.

We have also reported off-lesion EP values at 3 days after illumination, when morphological damage was at a peak.[12] The off-lesion EP values were found to be significantly depressed at all sites apical to the lesion (A-D) while remaining unchanged at sites basal to the lesion (E-H). In the present study, 2 weeks after illumination, the apical EP (sites A-D) was found to have returned to the normal control value, with no significant difference between different cochlear turns. Morphological examination revealed significant repair of the damaged structures; thus, the correlation between the EP and the morphological changes observed in previous short-term studies is seen to continue over the long term as well.

This depressant effect of a local lesion on the EP in the different cochlear turns apical to the lesion was first pointed out by Okumura.[18] He observed this effect after making repeated jabs with a glass electrode to the lateral wall of the guinea pig cochlea. He does not mention whether the effect was reversible. In the present study, the depressant effect was found to be transient and reversible, as are midlesion EP changes at 2 weeks.[13]

So far, we do not have a clear understanding as to why a localized lesion should cause a depression of the EP in the apical direction. This phenomenon cannot be explained as a result of the longitudinal flow of the endolymph. Salt and Thalmann[19] reported that the endolymph flowed basalward and that the flow rate was extremely slow (0.0036 mm/min). Nor are active oxygen species expected to act as a causative factor. They have an extremely short effective life span, and thus, their effects are expected to be confined to only a limited area around the lesion. One possibility may be that the greater number of strial marginal cells in the basal turn compared with that in the apical turn has some relationship to the EP.[20,21] Okumura[18] tried to explain apical-oriented EP depression as a difference in leakage current and electrical resistance based on this anatomic difference. Direct measurement of potassium ion or adaptation of some biochemical technique might be helpful in clarifying the mechanism involved in such EP changes, but so far, we have not had any success.

The situation seems to be different when damage to the stria is extensive. If the lesion is larger than those produced in the present experiment, or if lesions are multiple, the EP recovery will be hindered or the EP will stay at a depressed level throughout the entire cochlea. A decreased EP has been often reported in chronic strial disorders. Gratton et al[22] reported that the EP values for old Mongolian gerbils with marked atrophy of the stria vascularis were decreased in proportion to the degree of strial degeneration. A decreased EP was also reported for Wv (viable dominant spotting) mice with congenital absence of intermediate cells in the stria vascularis.[23]

After scrutinizing audiometric patterns and progression of symptoms over time in cases of sudden idiopathic sensorineural hearing loss, Mattox and Simmons[24] proposed that damage in the lower cochlear turn has a depressant effect on the sound perception mechanism lying apical to this. They concluded that hearing recovery was always better at lower frequencies than at higher frequencies. They assumed the damage in the lower turn to be a membrane rupture. They thought that low-frequency hearing loss, which is a common type of sudden hearing loss, is caused by this effect, and that recovery, which occurs in most cases within 2 weeks, is a result of healing of the membrane rupture.

So far, no morphological evidence has been presented regarding their membrane rupture theory, but it is interesting to note that their observations and proposed explanation of the apical-oriented depressant effect of a lesion in the lower turn and the reversibility of deafness within 2 weeks appear very similar to the course of EP change found in the present study. Although EP depression cannot be directly related to hearing loss, the EP has been shown by previous investigators to be essential for normal auditory function.[25-27] Sudden idiopathic sensorineural hearing loss as such may have various different causes, such as viral labyrinthitis[28] or inner ear hemorrhage.[29] Circulation disorders in the cochlear lateral wall similar to the present localized lesions may be responsible for a number of types of reversible sudden idiopathic hearing loss, especially when the loss is in the lower tone range.

ACKNOWLEDGMENT — We express our thanks to David Telke for his help in the preparation of this manuscript.

REFERENCES

1. Perlman HB, Kimura RS, Fernandez C. Experiments on temporary obstruction of the internal auditory artery. Laryngoscope 1959;69:591-613.

2. Suga F, Preston J, Snow JB. Experimental microembolization of cochlear vessels. Arch Otolaryngol 1970;92:213-20.

3. Kimura RS. Animal models of inner ear vascular disturbances. Am J Otolaryngol 1986;7:130-9.

4. Dietrich WD, Watson BD, Busto R, Ginsberg MD, Bethea JR. Photochemically induced cerebral infarction. Acta Neuropathol (Berl) 1987;72:315-25.

5. Nanda SK, Hatchell DL, Tiedeman JS, Dutton JJ, Hatchell MC, McAdoo T. A new method for vascular occlusion. Arch Ophthalmol 1987;105:1121-4.

6. Umemura K, Kohno Y, Matsuno H, Uematsu T, Nakashima M. A new model for photochemically induced thrombosis in the inner ear microcirculation and the use of hearing loss as a measure for microcirculatory disorders. Eur Arch Otorhinolaryngol 1990;248:105-8.

7. Foote CS. Photosensitized oxidation and singlet oxygen: consequences in biological systems. In: Pryor WA, ed. Free radicals in biological systems. New York, NY: Academic Press, 1976:85-133.

8. Watson BD, Dietrich WD, Busto R, Wachtel MS, Ginsberg MD. Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann Neurol 1985;17:497-504.

9. Iwasaki S, Mizuta K, Gao J, Wu R, Hoshino T. Focal microcirculation disorder induced by photochemical reaction in the guinea pig cochlea. Hear Res 1997;108:55-64.

10. Gao J, Hoshino T, Iwasaki S, Wu R. Photochemically induced focal cochlear lesions in the guinea pig. I. DAB staining and SEM study. Microsc Res Tech 1998;41:323-33.

11. Miyashita H, Iwasaki S, Hoshino T. Photochemically induced focal cochlear lesions in the guinea pig. II. A transmission electron microscope study. Microsc Res Tech 1998;41:334-40.

12. Wu R, Hoshino T. Changes in off-lesion endocochlear potential following localized lesion in the lateral wall. Acta Otolaryngol (Stockh) 1999;119:550-4.

13. Wu R, Hoshino T, Nagura M. Endocochlear potential in focal lesions of the guinea pig cochlea. Hear Res 1999;128:103-11.

14. Hallén O, McPherson DL, Axelsson A, Miller JM. Acute electrophysiological and morphological effects of mechanical trauma in the guinea pig cochlea. Acta Otolaryngol (Stockh) 1974;78:162-72.

15. Hallén O, McPherson DL, Axelsson A, Miller JM. Long-term morphological and electrophysiological effects of small mechanical lesions in the guinea pig cochlea. Acta Otolaryngol (Stockh) 1974;78:309-20.

16. Poje CP, Sewell DA, Saunders JC. The effects of exposure to intense sound on the DC endocochlear potential in the chick. Hear Res 1995;82:197-204.

17. Melichar I, Syka J, Ulehlova L. Recovery of the endocochlear potential and the K^+ concentrations in the cochlear fluids after acoustic trauma. Hear Res 1980;2:55-63.

18. Okumura M. Investigation of endocochlear potential at the different turns in the guinea pig cochlea [in Japanese]. J Osaka Med College 1996;55:34-63.

19. Salt AN, Thalmann R. Rate of longitudinal flow of cochlear endolymph. In: Nadol JB, ed. Meniere's disease. Amsterdam, the Netherlands: Kugler, 1989:69-73.

20. Santi PA, Lakhani B, Bingham C. The volume density of cells and capillaries of the normal stria vascularis. Hear Res 1983;11:7-22.

21. Lohuis PJFM, Patterson K, Rarey KE. Quantitative assessment of the rat stria vascularis. Hear Res 1990;47:95-102.

22. Gratton MA, Schmiedt RA, Schulte BA. Age-related decreases in endocochlear potential are associated with vascular abnormalities in the stria vascularis. Hear Res 1996;102:181-90.

23. Steel KP, Barkway C. Another role for melanocytes: their importance for normal stria vascularis development in the mammalian inner ear. Development 1989;107:453-63.

24. Mattox DE, Simmons FB. Natural history of sudden sensorineural hearing loss. Ann Otol Rhinol Laryngol 1977;86:463-80.

25. Offner FF, Dallos P, Cheatham MA. Positive endocochlear potential: mechanisms of production by marginal cells of stria vascularis. Hear Res 1987;29:117-24.

26. Salt AN, Melichar I, Thalmann R. Mechanisms of endocochlear potential generation by stria vascularis. Laryngoscope 1987;97:984-91.

27. Wangemann P, Liu J, Marcus DC. Ion transport mechanisms responsible for K^+ secretion and the transepithelial voltage across marginal cells of stria vascularis in vitro. Hear Res 1995;84:19-29.

28. Schuknecht HF, Kimura RS, Naufal PM. The pathology of sudden deafness. Acta Otolaryngol (Stockh) 1973;76:75-97.

29. Johnson A, Hawke M, Berger G. Sudden deafness and vertigo due to inner ear hemorrhage. A temporal bone case report. J Otolaryngol 1984;13:201-7.

LIGAND-GATED PURINERGIC RECEPTORS ARE DIFFERENTIALLY EXPRESSED IN THE ADULT RAT VESTIBULAR PERIPHERY

JAMES L. KREINDLER, MD
NEW YORK, NEW YORK

MARTA TROYANOVSKAYA, PHD
NEW YORK, NEW YORK

PHILLIP A. WACKYM, MD
MILWAUKEE, WISCONSIN

To further characterize the pattern of expression of the ligand-gated purinergic P2X receptors in the peripheral vestibular system, we conducted reverse transcription–polymerase chain reaction amplification of P2X1 and P2X2 messenger RNA extracted from adult rat vestibular ganglia (Scarpa's ganglia) and vestibular end organs. Transcripts encoding P2X1 were found in both Scarpa's ganglia and the end organs, but transcripts encoding P2X2 were found only in the vestibular end organs. These results support previous electrophysiological data, and they provide a more complete understanding of the specific role of purinergic (adenosine-5'-triphosphate) transmission in the vestibular periphery.

KEY WORDS — adenosine-5'-triphosphate, crista, efferent innervation, hair cell, macula, purinergic receptor, reverse transcription–polymerase chain reaction, Scarpa's ganglion, vestibular system.

INTRODUCTION

In the past several decades, our understanding of the vestibular system has expanded tremendously. This is especially true of the neuroanatomy and neurophysiology; however, much remains to be learned about the neurochemical basis of vestibular function. The sensory information transduced by the vestibular end organs is transmitted to the central vestibular system via the afferent fibers within the vestibular nerve. The efferent vestibular nerve fibers originate from neuronal cell bodies located within the brain stem and project out to the vestibular end organs, in which they innervate and modulate the afferent pathway. The mammalian vestibular hair cells differ in their afferent and efferent innervation patterns. Type I hair cells synapse with the primary afferent dendrites via calyceal specializations that surround the cell body. The type II hair cells synapse with the primary afferent dendrites by way of bouton endings on the basal and basolateral cell membrane. As a result of these differences, the efferent axons synapsing in the vestibular periphery have very different interactions with type I as opposed to type II hair cells. For the type I hair cell, the vast majority of efferent terminals are located on the calyceal ending of the afferent dendrite, making the efferent innervation postsynaptic. For the type II hair cell, the majority of efferent terminals are located on the sensory cell itself, making the efferent innervation presynaptic.

The major efferent neurotransmitter is acetylcholine[1]; however, this cholinergic input is received by a diverse group of muscarinic[2,3] and nicotinic[4,5] acetylcholine receptors. In addition, there is evidence that other neuromodulators and neurotransmitters, such as calcitonin gene–related peptide[6] and adenosine-5'-triphosphate (ATP), are part of the efferent vestibular system and thereby modulate afferent function.[7,8] The focus of this study is to determine the distribution of 2 ligand-gated ATP receptors, termed P2X1 and P2X2, within the vestibular end organs and primary afferent ganglia (Scarpa's ganglia).

The intracellular role of ATP is well established; however, the role of ATP as an energy carrier is one of a myriad of functions performed by this molecule. The extracellular actions of ATP and its major breakdown product, adenosine, have been studied since the early 20th century, beginning with its potent effects on cardiac vasculature. By the mid-1970s, ATP had been identified as an extracellular effector in numerous sites, including nervous tissue, endothelium, smooth muscle, hepatocytes, and individual cells of the hematopoetic and immunologic cell lines (for re-

---

From the Department of Pediatrics, College of Physicians and Surgeons, Columbia University (Kreindler), and the Department of Otolaryngology, Mount Sinai School of Medicine (Troyanovskaya), New York, New York, and the Department of Otolaryngology and Communication Sciences, Medical College of Wisconsin, Milwaukee, Wisconsin (Wackym). This research was supported by National Institutes of Health/National Institute on Deafness and Other Communication Disorders grant DC02971 (P.A.W.). This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq;); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Medical College of Wisconsin.

CORRESPONDENCE — Phillip A. Wackym, MD, Dept of Otolaryngology and Communication Sciences, Medical College of Wisconsin, 9200 W Wisconsin Ave, Milwaukee, WI 53226.

view, see Burnstock[9,10]).

Adenosine-5'-triphosphate exerts its effects at the cell membrane through binding to specific receptors. These purinergic receptors may be classified into P1 and P2 subtypes, according to the comparative binding properties of different agonists.[9,10] The P1 receptors all are guanine nucleotide binding protein (G-protein)–linked, they all activate adenylyl cyclase, and they all are most responsive to adenosine. Conversely, P2 receptors, which may be either G-protein–linked or ligand-gated ion channels, do not activate adenylyl cyclase and are activated preferentially by ATP.[9,10] More recently, unique P1 and P2 receptor subtypes have been identified on the basis of different pharmacological and electrophysiological activity.[11] The P1 receptors are classified as P1A1, P1A2a, P1A2b, and P1A3,[12] and the P2 receptors are classified as P2X, P2Y, P2U, P2T, and P2Z.[11]

The P2X receptors constitute a family of ligand-gated, cation-selective ion channels.[13] Cloning experiments currently define 7 P2X receptors with evidence for splice variation.[14-22] Binding of ATP to these receptors results in measurable currents in the millisecond range in oocytes injected with P2X messenger RNA (mRNA).[21] Activation of these channels results in cellular depolarization and possibly in the release of intracellular calcium stores.[13] Electrophysiological studies have demonstrated that P2X subtypes differ with respect to desensitization after repeated stimulation with ATP (for review, see Buell et al[23]). All P2X receptors are noncompetitively antagonized by suramin and pyridoxylphosphate-6-azophenyl-2', 4'-disulfonic acid (PPADS; for review, see Surprenant et al[22]), except for the recently cloned P2X4 and P2X6.[23,24] Three functional groups of P2X have been proposed by Collo et al[24]: 1) P2X1 and P2X3 (very rapid desensitization and sensitivity to α,β-methylene-ATP [α,β-meATP]); 2) P2X2 and P2X5 (sensitive to the antagonists suramin and PPADS); 3) and P2X4 and P2X6 (not sensitive to antagonism with suramin and PPADS).

Although the exact structure of the P2X family remains unknown, evidence supports an approximately 350– to 400–amino acid molecule with intracellular carboxyl and amino termini and 1 large extracellular domain.[20] This large extracellular domain contains conserved regions of hydrophobicity, as well as 10 conserved cysteines and 13 conserved glycines.[24] Secondary structure predictions elucidate a conserved pattern of small, hydrophobic amino acids along one side of the helix that may form part of the ion pore.[24] This structure is unique, in contrast to more traditional ligand-gated ion channels, such as the nicotinic acetylcholine receptor.[20]

Among the many cell types in which ATP has been identified as a neurotransmitter are the hair cells of the guinea pig cristae.[13] The ATP is thought to act on these hair cells by activation of nonselective cation channels and the release of intracellular calcium stores.[13] Studies of isolated frog semicircular canals have demonstrated that ATP acts on a receptor that is nondesensitizing and is not stimulated by α,β-meATP.[7] This pharmacological profile suggests the presence of the P2X subtype in the vestibular periphery.[8]

This study used reverse transcription–polymerase chain reaction (RT-PCR) in order to characterize the expression of the ligand-gated P2X1 and P2X2 receptors in the vestibular periphery. A microdissection strategy was used to separate the end organs from the ganglia, because the mRNA in Scarpa's ganglia represents primary afferent neurons (mRNA would be located within the somata rather than in the dendrites or axons), Schwann cells, and connective tissues; while the mRNA isolated from the end organs represents type I and type II hair cells, supporting cells, and connective tissues.

MATERIALS AND METHODS

The experimental design adhered to the NIH *Guide for the Care and Use of Laboratory Animals* and the Medical College of Wisconsin Animal Use Committee.

Tissue Preparation. After anesthesia with CO_2, 30 Sprague-Dawley rats were painlessly decapitated. The experiments were replicated 3 times, and therefore, a total of 90 rats were used. The temporal bones were rapidly harvested, and the vestibular end organs and ganglia were microdissected from the bony labyrinth and processed separately. The survey tissues included rat total brain homogenate (positive control). Total RNA and mRNA were isolated with a Purescript RNA Isolation kit (Gentra Systems, Inc, Minneapolis, Minn) and a Micro Fast Track kit (Invitrogen, San Diego, Calif). Reverse transcription of the RNA to generate complementary DNA (cDNA) was accomplished with AMV reverse transcriptase, with oligo-dT used as a primer (cDNA Cycle Kit, Invitrogen).

Polymerase Chain Reaction. Four PCR primers were synthesized according to previously published material,[25] and in addition, 2 PCR nested sense primers were designed (see Table). A total of 2 to 4 µL of cDNA was used for PCR amplification in the presence of 2.5 U of Taq DNA polymerase (Perkin-Elmer-Cetus, Norwalk, Conn). Thermal cycling was performed with the following conditions for the P2X1 primers: 94°C for 1 minute, 57°C for 1 minute, and 72°C for 2 minutes (for 30 cycles), followed by 7

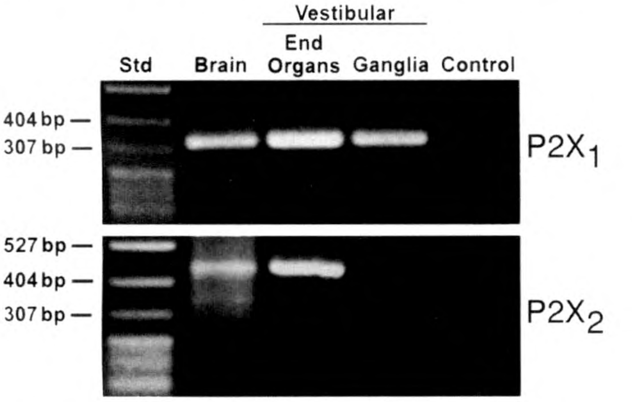
POLYMERASE CHAIN REACTION PRIMERS USED FOR IDENTIFICATION OF P2X1 AND P2X2 IN ADULT RAT BRAIN, PERIPHERAL VESTIBULAR GANGLION, AND VESTIBULAR END ORGAN MESSENGER RNA

Subunit	*Primer*	*Primer Sequence*	*Size**
P2X1	Sense	5'-TCTCTTATTGCTCCACATCC-3'	320 bp
P2X1	Antisense	5'-ATTGGTGAAAACTGGAGGAG-3'	
P2X1	Sense (nested)	5'-AGTTCAAATATGCCGAGGAC-3'	
P2X2	Sense	5'-GCATGGACAGGCAGGGAAAT-3'	439 bp
P2X2	Antisense	5'-AGAGGTGAGCAGGGAAAGGT-3'	
P2X2	Sense (nested)	5'-TGGGAGAAGGGGCAGAGC-3'	

*Size in base pairs of expected amplified nucleic acid product.

minutes at 72°C (extension) in a model 2400 thermocycler (Perkin-Elmer-Cetus). For the P2X2 primers, the thermal cycling conditions were 94°C for 1 minute, 59°C for 1 minute, and 72°C for 2 minutes (for 30 cycles), followed by 7 minutes at 72°C (extension) in a model 2400 thermocycler (Perkin-Elmer-Cetus). The PCR products were then separated on 1.0% agarose gels and stained with ethidium bromide, and the bands were visualized with ultraviolet illumination. The negative controls included amplification without templates. For increased specificity, nested primers were created for both P2X1 and P2X2 receptors (see Table). The second round of amplification was performed with the nested sense primer and the original antisense primer under conditions identical to those of the first round. All primers were synthesized by GenSet, Inc (La Jolla, Calif).

Cloning and Sequence Analysis. The PCR-amplified products were subcloned into the TA cloning vector (Invitrogen) and used for transformation of INVαF' competent cells. Positive transformants containing plasmids with inserts were selected by growing bacteria on LB plates containing 50 µg/mL of kanamycin and X-Gal. Plasmids were isolated from the minicultures and digested with *Eco*RI. Plasmids containing the PCR product were then sequenced in the core sequencing facility at the Utah State Biotechnology Center (Utah State University, Logan, Utah) by means of a model 373 Stretch Sequencing System (Perkin-Elmer-Cetus). A computer analysis of the sequence data was carried out with the Vector NTI Suite (InfoMax, Inc, North Bethesda, Md) software program.



Reverse transcription–polymerase chain reaction amplification of purinergic P2X1 and P2X2 receptor messenger RNAs after 2 rounds of polymerase chain reaction. Lane 1 — molecular weight standard (Std; pBR322 DNA-Msp I Digest, New England Biolabs, Beverly, Mass); lane 2 — P2X1 and P2X2 in brain (positive control); lane 3 — P2X1 and P2X2 in vestibular end organs; lane 4 — P2X1 in ganglia; lane 5 — negative control.

RESULTS

Polymerase chain reaction products of the predicted sizes were obtained (see Figure), and their identity to published P2X1 and P2X2 receptors was confirmed by sequence analysis.[25,26] As shown in the Figure, in adult rats, the P2X1 receptor was expressed in both peripheral vestibular ganglia and vestibular end organs. In distinction to this, P2X2 expression was limited to the vestibular end organs. Although 1 isoform of P2X1 was found, 4 different isoforms of P2X2 were expressed within the vestibular end organs.[26]

Expression of P2X1 in the adult rat brain and the peripheral vestibular system was of a low level, though similar in the two tissues (see Figure). This level of expression was considered low because the expected PCR products were only visualized after a second round of PCR. On the basis of the qualitative differences in the intensity of PCR products after electrophoretic separation and the differential ease in amplification, P2X2 was found to have higher levels of expression in the vestibular end organs than in the adult rat brain, which is known to have lower levels of expression than other P2X receptors.[24] The expected P2X2 PCR products were only visualized after a second round of PCR in amplification of brain cDNA, while it was possible to detect P2X2 cDNA after 1 round of PCR in amplification of vestibular end organ cDNA. However, a second round of PCR was required to produce a robust level of amplification (see Figure).

DISCUSSION

The expression of P2X1 and P2X2 receptor mRNA in the adult rat vestibular end organs confirms the work of Housley and Ryan[25]; however, the present study expanded this observation by demonstrating that Scarpa's ganglia expresses P2X1, but not P2X2, receptor mRNA (see Figure). In contrast, within the cochlea, P2X2 mRNA is expressed in the adult rat spiral ganglion.[25]

Although no transcript for P2X2 receptor was found in the vestibular primary afferent neurons (Scarpa's ganglia), partial cDNAs encoding 4 splice variants of the P2X2 receptor have been isolated from the vestibular end organs.[26] In all 4 cDNAs, the deletions were of different lengths, but started at the same position on the P2X2 gene (Val-370 codon) located toward the intracellular carboxyl terminus. One of these receptor isoforms was identical in sequence to the recently published P2X2(b) receptor.[27] The remaining 3 novel splice variants of the P2X2 receptor were designated P2X2(e), P2X2(f), and P2X2(g) (GenBank accession numbers AF028603, AF028604, and AF028605, respectively). Functional expression has been reported for only the P2X2(b) splice variant.[27] When antisense (complementary RNA) oligonucleotides for this isoform of P2X2 were microinjected into *Xenopus laevis* oocytes or mammalian HEK 293 cells, there was significantly lower antagonist sensitivity and more rapid desensitization than with the original P2X2(a). This effect may be determined by the deleted proline- and serine-rich segments and/or phosphorylation motifs from the tail region that result in the formation of the P2X2(b) subunit.[27] The functional significance of the P2X2(b) and the 3 novel isoforms P2X2(e), P2X2(f), and P2X2(g) of the P2X2 receptor within the vestibular end organs is unknown. Further investigation by means of physiological and neuroanatomical techniques such as in situ hybridization will help resolve these issues in the vestibular end organs.

There is no evidence that there are separate purinergic efferent vestibular axons; however, according to studies of the neuromuscular junction, it is possible that ATP is colocalized with acetylcholine in the same neurons and, most likely, in the same synaptic vesicles.[28-30] Alternatively, a neurohumoral mechanism of stimulation of P2X1 and P2X2 receptors is possible, because the levels of ATP in the endolymph average 13 nmol/L.[31]

Stimulation of the vestibular efferent axons results in complex effects on primary afferent activity. These include rapid, short-lived excitatory effects; delayed, prolonged inhibitory effects; and complex efferent effects.[32-35] After initial characterization of the primary afferents as being excited or inhibited by electrical activation of efferent fibers, the effect of ATP on afferent activity was studied by Rossi et al.[35] Perilymphatic application of ATP increased the rate of hair cell excitatory postsynaptic potentials in afferents that were also excited by efferent stimulation, while there was no effect on afferents showing efferent inhibition. Interestingly, these ATP responses were transient in nature, and once they disappeared, the afferents were no longer affected by electrical stimulation of the efferent axons.

There is neurophysiological evidence that mammalian vestibular type I and type II hair cells contain P2X receptors and that application of ATP results in a rapid inward current.[13] The P2X receptors in hair cells, like those found elsewhere,[36] open a nonselective cation channel that appreciably slows the permeability to Ca^{2+}.[37] The ATP-induced inward current will depolarize the hair cell and is associated with a rapid rise in intracellular free Ca^{2+}.[13,38,39] A second, slower rise in intracellular Ca^{2+} takes place even in the absence of extracellular Ca^{2+},[38,39] suggesting the presence of a P2Y or some other metabotropic receptor. Since Ca^{2+} metabolism is integral to signal transduction by hair cells,[40] efferent modulation of vestibular afferent function via Ca^{2+} regulatory mechanisms may prove to be just as important as efferent cholinergic modulation. Physiological and binding studies of cochlear outer hair cells suggest that the P2X receptors are located at the apical surface.[11,25,38,41-43] It is therefore possible that there is a P2Y efferent receptor in the cochlea and that P2X1 and P2X2 are efferent receptors on type II hair cells, while P2X1 is an efferent receptor on the calyces surrounding the type I hair cells. In addition, it is possible that ATP could have other functions in efferent modulation besides depolarizing hair cells and increasing cytosolic Ca^{2+}, should it be demonstrated to be an efferent neurotransmitter. In the context of other cholinergic synapses, ATP has been shown to modulate transmission by directly affecting nicotinic receptors[44] or by phosphorylating nicotinic receptors via second messengers.[45,46]

The data presented herein suggest that in the adult rat vestibular periphery, P2X1 and P2X2 receptors in the vestibular end organs and P2X1 receptors in Scarpa's ganglia may be potential targets for the pharmacotherapy of peripheral vestibular disorders. Further cellular, molecular, and physiological characterization of these receptors within the vestibular periphery is necessary for exploring this possibility.

REFERENCES

1. Goldberg JM, Brichta AM, Wackym PA. Efferent vestibular system: anatomy, physiology and neurochemistry. In: Beitz A, Anderson J, eds. Neurochemistry of the vestibular system. Boca Raton, Fla: CRC Press, 2000:61-94.

2. Wackym PA, Chen CT, Ishiyama A, Pettis RM, López I, Hoffman L. Muscarinic acetylcholine receptor subtype mRNAs in the human and rat vestibular periphery. Cell Biol Int 1996; 20:187-92.

3. Ishiyama A, López I, Wackym PA. Molecular characterization of muscarinic receptors in the human vestibular periphery: implications for pharmacotherapy. Am J Otol 1997;18:648-54.

4. Wackym PA, Popper P, López I, Ishiyama A, Micevych PE. Expression of α4 and β2 nicotinic acetylcholine receptor subunit mRNA and localization of α-bungarotoxin binding proteins in the rat vestibular periphery. Cell Biol Int 1995;19:291-300.

5. Anderson AD, Troyanovskaya M, Wackym PA. Differential expression of α2-7, α9 and β2-4 nicotinic acetylcholine receptor subunit mRNA in the vestibular end-organs and Scarpa's ganglia of the rat. Brain Res 1997;778:414-8.

6. Wackym PA. Ultrastructural organization of calcitonin gene–related peptide immunoreactive efferent axons and terminals in the rat vestibular periphery. Am J Otol 1993;14:41-50.

7. Aubert A, Norris CH, Guth PS. Influence of ATP and ATP agonists on the physiology of the isolated semicircular canal of the frog (*Rana pipiens*). Neuroscience 1994;62:963-74.

8. Evans RJ, Lewis C, Buell G, Valera S, North RA, Surprenant A. Pharmacologic characterization of heterologously expressed ATP-gated cation channels (P2x purinoceptors). Mol Pharmacol 1995;48:178-83.

9. Burnstock G. Purinergic mechanisms. Ann N Y Acad Sci 1990;603:1-17.

10. Burnstock G. Purinergicotransmission. Brain Res Bull 1999;50:355-7.

11. Mockett BG, Bo X, Housley GD, Thorne PR, Burnstock G. Autoradiographic labelling of P2 purinoceptors in the guinea-pig cochlea. Hear Res 1995;84:177-93.

12. Ford MS, Maggirwar SB, Rybak LP, Whitworth C, Ramkumar V. Expression and function of adenosine receptors in the chinchilla cochlea. Hear Res 1997;105:130-40.

13. Rennie KJ, Ashmore JF. Effects of extracellular ATP on hair cells isolated from the guinea-pig semicircular canals. Neurosci Lett 1993;160:185-9.

14. Surprenant A, Rassandren F, Kawashima E, North RA, Buell G. The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7). Science 1996;272:735-8.

15. Soto F, Garcia-Guzman M, Karschin C, Stuhmer W. Cloning and tissue distribution of a novel P2X receptor from rat brain. Biochem Biophys Res Commun 1996;223:456-60.

16. Garcia-Guzman M, Soto F, Laube B, Stuhmer W. Molecular cloning and functional expression of a novel rat heart P2X purinoceptor. FEBS Lett 1996;388:123-7.

17. Soto F, Garcia-Guzman M, Gomez-Hernandez JM, Hollmann M, Karschin C, Stuhmer W. P2X4: an ATP-activated ionotropic receptor cloned from rat brain. Proc Natl Acad Sci U S A 1996;93:3684-8.

18. Chen CC, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G, Wood JN. A P2X purinoceptor expressed by a subset of sensory neurons. Nature 1995;377:428-31.

19. Housley GD, Greenwood D, Bennett T, Ryan AF. Identification of a short form of the P2xR1-purinoceptor subunit produced by alternative splicing in the pituitary and cochlea. Biochem Biophys Res Commun 1995;212:501-8.

20. Brake AJ, Wagenbach MJ, Julius D. New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. Nature 1994;371:519-23.

21. Valera S, Hussy N, Evans RJ, et al. A new class of ligand-gated ion channel defined by P2X receptor for extracellular ATP. Nature 1994;371:516-9.

22. Surprenant A, Buell G, North RA. P2X receptors bring new structure to ligand-gated ion channels. Trends Neurosci 1995;18:224-9.

23. Buell G, Lewis C, Collo G, North RA, Surprenant A. An antagonist-insensitive P2X receptor expressed in epithelia and brain. EMBO J 1996;1:55-62.

24. Collo G, North RA, Kawashima E, et al. Cloning of P2X5 and P2X6 receptors and the distribution and properties of an extended family of ATP-gated ion channels. J Neurosci 1996; 16:2495-507.

25. Housley GD, Ryan AF. Cholinergic and purinergic neurohumoral signalling in the inner ear: a molecular physiological analysis. Audiol Neurootol 1997;2:92-110.

26. Troyanovskaya M, Wackym PA. Evidence for three additional P2X2 purinoceptor isoforms produced by alternative splicing in the adult rat vestibular end-organs. Hear Res 1998; 126:201-9.

27. Simon J, Kidd EJ, Smith FM, et al. Localization and functional expression of splice variants of the P2X2 receptor. Mol Pharmacol 1997;52:237-48.

28. Dowdal MJ, Boyne AF, Whittaker VP. Adenosine triphosphate, a constituent of cholinergic synaptic vesicles. Biochem J 1972;140:1-12.

29. Schweitzer E. Coordinated release of ATP and ACh from cholinergic synaptosomes and its inhibition by calmodulin antagonists. J Neurosci 1987;7:2948-56.

30. Silinsky EM. On the association between transmitter secretion and the release of adenine nucleotides from mammalian motor nerve terminals. J Physiol (Lond) 1975;247:145-62.

31. Muñoz D, Thorne P, Housley G, Billett T. Adenosine 5'-triphosphate (ATP) concentrations in the endolymph and perilymph of the guinea pig cochlea. Hear Res 1995;90:119-25.

32. Dickman JD, Correia MG. Bilateral communication between vestibular labyrinths in pigeons. Neuroscience 1993;57: 1097-108.

33. Brichta AM, Goldberg JM. Afferent and efferent responses from morphological fiber classes in the turtle posterior crista. Ann N Y Acad Sci 1996;781:183-95.

34. Goldberg JM, Fernández C. Efferent vestibular system in the squirrel monkey: anatomical location and influence on afferent activity. J Neurophysiol 1980;43:986-1025.

35. Rossi ML, Martini M, Pelucchi B, Fesce R. Quantal nature of synaptic transmission at the cytoneural junction in the frog labyrinth. J Physiol (Lond) 1994;478:17-35.

36. Bean BP. Pharmacology and electrophysiology of ATP-activated ion channels. Trends Pharmacol Sci 1992;13:87-90.

37. Nakagawa T, Akaike N, Kimitsuki T, Komune S, Arima T. ATP-induced current in isolated outer hair cells of guinea pig cochlea. J Neurophysiol 1990;63:1068-74.

38. Ashmore JF, Ohmori H. Control of intracellular calcium by ATP in isolated outer hair cells of the guinea-pig cochlea. J Physiol (Lond) 1990;428:109-31.

39. Shigemoto T, Ohmori H. Muscarinic agonists and ATP

increase the intracellular Ca^{2+} concentration in chick cochlear hair cells. J Physiol (Lond) 1990;420:127-48.

40. Jaramillo F. Signal transduction in hair cells and its regulation by calcium. Neuron 1995;15:1227-30.

41. Housley GD, Greenwood D, Ahmoe JF. Localization of cholinergic and purinergic receptors in outer hair cells isolated from the guinea pig cochlea. Proc R Soc Lond B 1992;249:265-73.

42. Mockett BG, Housley GD, Thorne PR. Fluoresence imaging of extracellular purinergic receptor sites and putative ecto-ATPase sites on isolated cochlear hair cells. J Neurosci 1994;14: 6992-7007.

43. Housley GD. Extracellular nucleotide signaling in the inner ear. Mol Neurobiol 1998;16:21-48.

44. Nakazawa K. ATP-activated current and its interaction with acetylcholine-activated current in rat sympathetic neurons. J Neurosci 1994;14:740-50.

45. Fu WM. Potentiation by ATP of the postsynaptic acetylcholine response at developing neuromuscular synapses in *Xenopus* cell cultures. J Physiol (Lond) 1994;477:449-58.

46. Lu Z, Smith DO. Adenosine 5'-triphosphate increases acetylcholine channel opening frequency in rat skeletal muscle. J Physiol (Lond) 1991;436:45-56.

Ann Otol Rhinol Laryngol 110:2001

EFFECT OF FUROSEMIDE ON BASAL LAMINA ANIONIC SITES IN GUINEA PIG LABYRINTH

MITSUYA SUZUKI, MD KIMITAKA KAGA, MD

TOKYO, JAPAN

The authors studied the effects of acute furosemide administration on the basal lamina (BL) anionic sites in the stria vascularis, ampullar crista, and endolymphatic sac by using cationic polyethyleneimine (PEI). Furosemide was intravenously administered to albino guinea pigs with normal Preyer's reflexes. After 20 minutes, the bony labyrinth was removed and processed for histologic evaluation. Under a transmission electron microscope, a marked enlargement of the intercellular spaces was observed in the stria vascularis. The PEI distribution decreased significantly on the capillary BL in the stria vascularis and on the subepithelial BL in the sensory, transitional, and dark cell areas. However, no significant change was observed on the capillary BL or the subepithelial BL in the endolymphatic sac. These findings suggest that acute furosemide administration severely alters the distribution of the anionic sites in the strial capillary BL and in the subepithelial BL in the ampullar crista, but not in the capillary BL or the subepithelial BL of the endolymphatic sac.

KEY WORDS — anionic site, basal lamina, furosemide, labyrinth, polyethyleneimine.

INTRODUCTION

The blood-labyrinth barrier, which is divided into the size barrier and the negative charge barrier, plays an important role in endolymph production and absorption.[1-4]

Basal lamina (BL) anionic sites are thought to act as negative charge barriers that contribute to the selective permeability of electrically charged macromolecules and ions in the labyrinth.[2-6] Cationic tracers, such as ruthenium red and polyethyleneimine (PEI), are useful in observing localization of the BL anionic sites in the labyrinth.[2-7]

The stria vascularis and the ampullar dark cell area are thought to be the principal endolymph production sites.[1,8] Since endolymphatic hydrops can be experimentally induced by the obliteration of the endolymphatic sac in a normal guinea pig, the endolymphatic sac is thought to be a main endolymph absorption site.[9] Therefore, a negative charge barrier function in these sites may be important in maintaining the specific chemical composition of endolymph and in maintaining cochlear homeostasis or vestibular function.

Administration of furosemide causes an edematous change in the intercellular spaces of the intermediate cell region in the stria vascularis.[10] It has been reported that a patient with ototoxicity due to loop diuretics had dilatation of the intercellular fluid spaces in the stria vascularis and the dark cell area.[11] Therefore, it was suggested that a permeability change in the stria vascularis and the dark cell area is caused by administration of loop diuretics. Rybak[12] speculated that furosemide may alter the endolymph-perilymph barrier. Futaki et al[13] found that an increase in caloric response in typical Meniere's disease is induced by systemically administered furosemide. It was suspected that systemically administered furosemide may alter the blood-labyrinth barrier in the endolymphatic sac, the stria vascularis, or the ampullar crista, resulting in reduction of endolymphatic hydrops and an increase in caloric response.

To investigate the effect of furosemide on the blood-labyrinth charge barrier, we treated guinea pigs with intravenous furosemide and observed subsequent changes in the localization of BL anionic sites of the stria vascularis, and of the sensory, transitional, and dark cell areas of the ampullar crista and the endolymphatic sac.

MATERIALS AND METHODS

Animals. Twelve Hartley strain guinea pigs (250 to 300 g) with a normal Preyer's reflex were divided equally into control and treated groups. All experiments were performed in accordance with the guidelines of the Declaration of Helsinki.

Furosemide Administration and Tissue Preparation. After intraperitoneal injection of pentobarbital sodium (30 mg/kg of weight), control guinea pigs were administered physiological saline solution (8 mL/kg of body weight) adjusted to pH 7.4 with sodium hydroxide for 1 minute via an axial vein under the surgical microscope. By the same procedure, each animal in the treated group was injected with furo-

---

From the Department of Otolaryngology, University of Tokyo, Tokyo, Japan.

CORRESPONDENCE — Mitsuya Suzuki, MD, Dept of Otolaryngology, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo, 113 Japan.

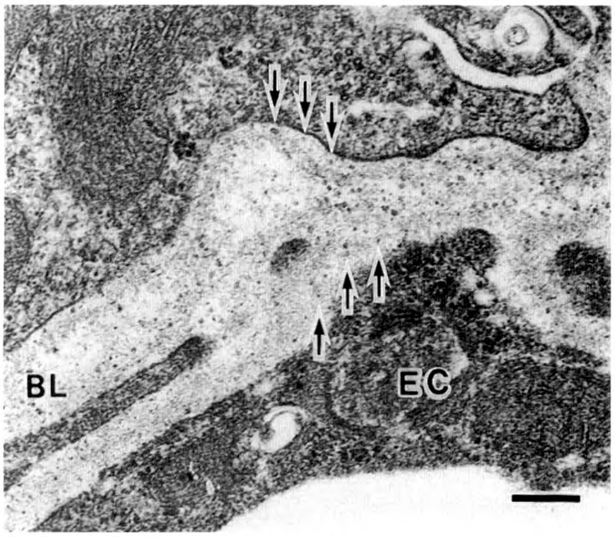


Fig 1. Polyethyleneimine (PEI) distribution (arrows) on capillary basal lamina (BL) in stria vascularis (control animal). EC — endothelial cell, bar — 0.2 μm.

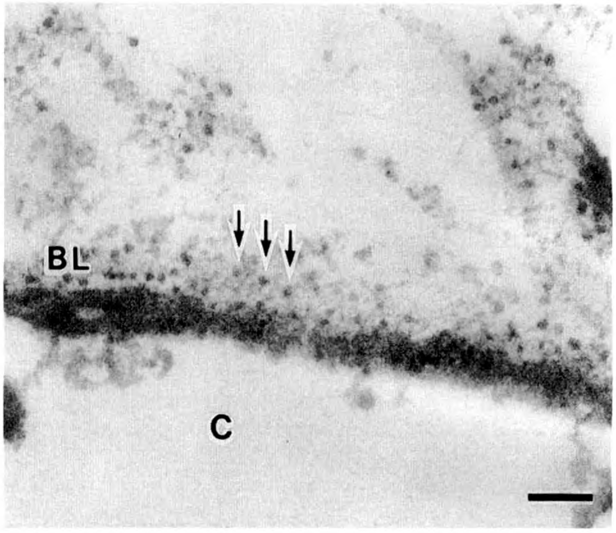


Fig 2. PEI particles (arrows) on capillary BL in endolymphatic sac (control animal). C — capillary lumen, bar — 0.2 μm.

semide solution of 8 mL/kg of body weight (10 mg/mL). All animals were then covered with a blanket to prevent hypothermia. Twenty minutes later, the animals were painlessly decapitated and both temporal bones were removed. A small hole was made in the cochlear apex, the stapes was removed, and the round window was opened. Each bony labyrinth was rinsed with 0.1-mol/L cacodylate buffer for 1 minute and then immersed for 15 minutes in a 0.5% PEI solution (molecular weight, 1,800; Polysciences, Warrington, Pa) dissolved in 0.1-mol/L cacodylate buffer adjusted to pH 7.4 with hydrochloric acid. Each bony labyrinth was rinsed again in 0.1-mol/L cacodylate buffer for a few minutes in order to wash out any remaining PEI solution. Cacodylate buffer was used in this study because phosphate buffer can precipitate PEI.[14] All bony labyrinths were immersed in 2% phosphotungstic acid and 2.5% glutaraldehyde at 4°C for 3 hours to stain the PEI particles and fix the tissue. Each bony labyrinth was immersed for decalcification in 10% ethylenediaminetetraacetic acid solution, in which it was left for 2 weeks. After this decalcification procedure, the bony labyrinth was rinsed with 0.1% cacodylate buffer and postfixed in 1% osmium tetroxide at 4°C for 2 hours.

The cochlea, ampullar crista, and endolymphatic sac were removed from each bony labyrinth. The cochleae were cut into 2 parts along the modiolus. All specimens were dehydrated through a series of graded alcohol, treated with propylene oxide, and embedded in epoxy resin (Quetol 812, Nishin, EM, Tokyo, Japan). Ultrathin sections of all specimens were double-stained and then examined with a Hitachi H 800 electron microscope.

Data Analysis. We analyzed the distribution of PEI particles on the capillary BL in the stria vascularis, on the subepithelial BL of sensory, transitional, and dark cell areas in the ampullar crista, and on the capillary and subepithelial BL of the endolymphatic sac. To analyze the changes in PEI distribution of each area as accurately as possible, we evaluated the number and size of the PEI particles, using rasters 5 mm wide. One tissue block was randomly selected from the stria vascularis, ampullar crista, and endolymphatic sac in each of the 24 ears studied. In these samples, PEI particles were examined on the capillary BL in either the stria vascularis or the endolymphatic sac and on the subepithelial BL in either the ampullar crista or the endolymphatic sac. Several photographs (magnification, ×48,000) were taken of each region of each animal. From these, 2 photographs were randomly selected to determine the number and diameter of PEI particles on a 2-μm BL segment. Thus, 12 photographs (12 unit lengths) were evaluated for each anatomic region in each group.

The area of PEI per 2 μm of BL for each anatomic region of each group was calculated as the mean of the sum total of [(radius of PEI particles)$^2\pi \times$ the number of PEI particles of each size] per 2 mm of BL (ie, PEI area).

Statistical Analysis. Values are reported as mean ± standard deviation. The area of PEI in the treated group was compared with that in the control group in each anatomic region by Student's *t*-test. A p value of <.05 was considered significant.

RESULTS

Control Animals. Neither shrinkage of intermediate cells nor enlargement of intercellular spaces was observed in the stria vascularis, the ampullar crista,

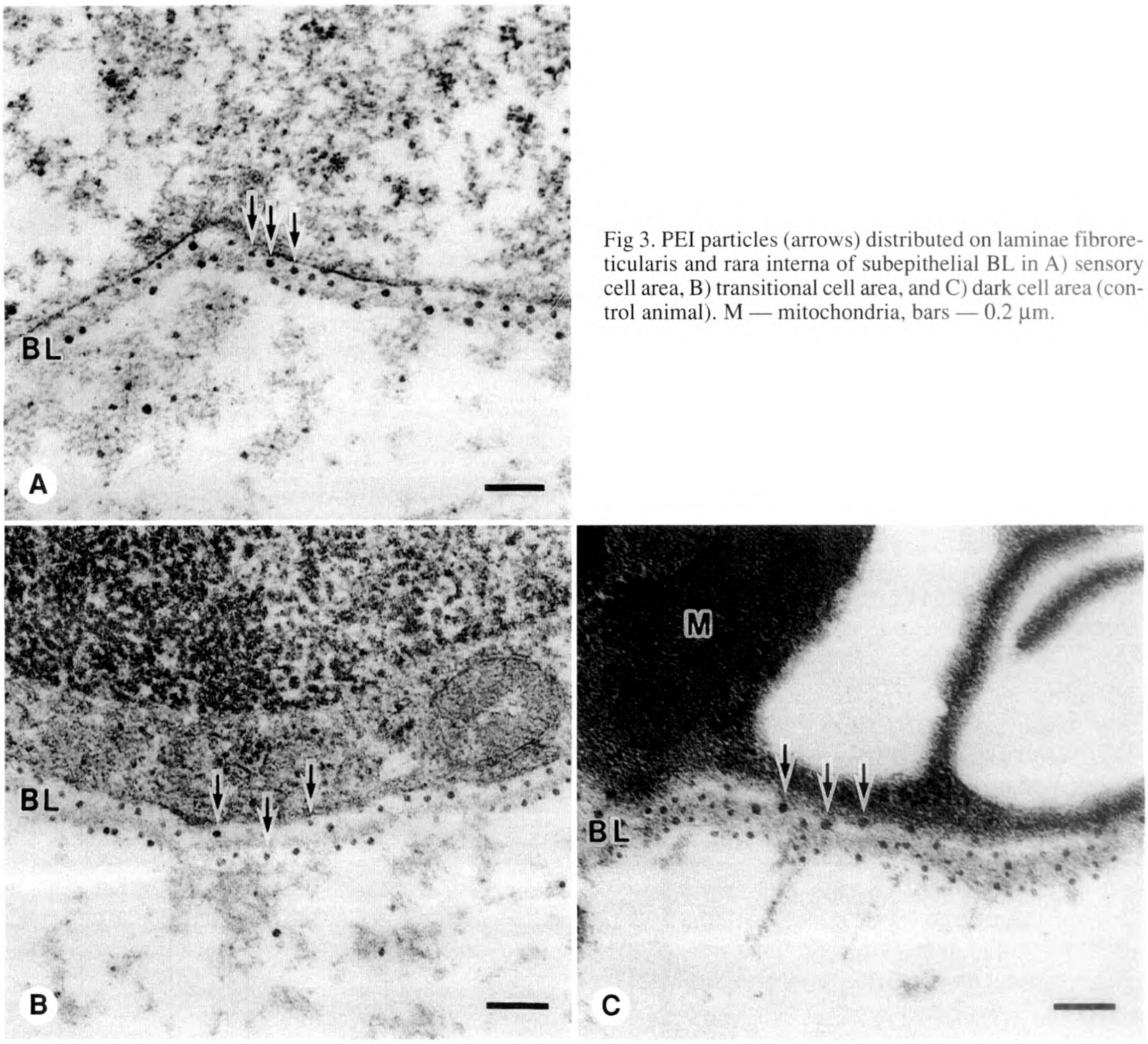


Fig 3. PEI particles (arrows) distributed on laminae fibroreticularis and rara interna of subepithelial BL in A) sensory cell area, B) transitional cell area, and C) dark cell area (control animal). M — mitochondria, bars — 0.2 μm.

or the endolymphatic sac in the control animals. We found PEI particles distributed on the laminae rarae externa and interna of the capillary BL in the stria vascularis (Fig 1). Some PEI particles were also observed in the pinocytotic vesicles of the endothelial cells in the stria vascularis. In the endolymphatic sac, PEI particles were observed on the capillary BL (Fig 2). A regular distribution of PEI particles was also observed on the laminae fibroreticularis and rara interna of the subepithelial BL in the sensory, transitional, and dark cell areas and in the endolymphatic sac (Fig 3). The area of PEI particles in the stria vascularis was smaller than that in the subepithelial BL in the ampullar crista and endolymphatic sac.

Treated Animals. In treated animals, severe enlargement of intercellular spaces and shrinkage of intermediate cells was noted (Fig 4). On the capillary BL of the stria vascularis, the PEI area was significantly different from that in the control animals (Figs 5 and 6).

In the ampullar crista, no marked edematous change was observed in the sensory, transitional, or dark cell areas. The PEI distributions on the subepithelial BL in those areas differed significantly between the treated and control animals (Figs 6 and 7).

A slight enlargement in the intercellular spaces was observed in the rugose portion of the endolymphatic sac. No significant difference in PEI distribution between the treated and control animals was observed on the subepithelial BL or the capillary BL in the endolymphatic sac (Figs 6 and 8).

No PEI particles were observed on the endolymphatic luminal surface of the endolymphatic space in any animal of either group.

DISCUSSION

This study evaluated changes in PEI distribution on the BL in the stria vascularis, sensory cells, transitional cells, and dark cell areas in the ampullar crista

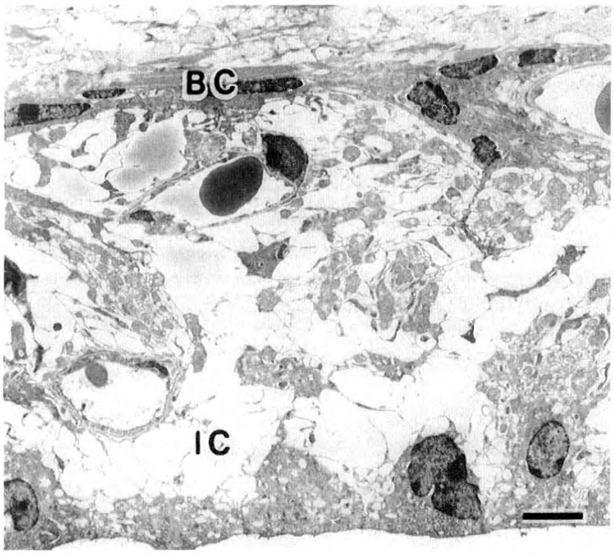


Fig 4. Severe enlargement of intercellular spaces and shrinkage of intermediate cells following administration of furosemide. IC — intercellular space, BC — basal cell, bar — 5 µm.

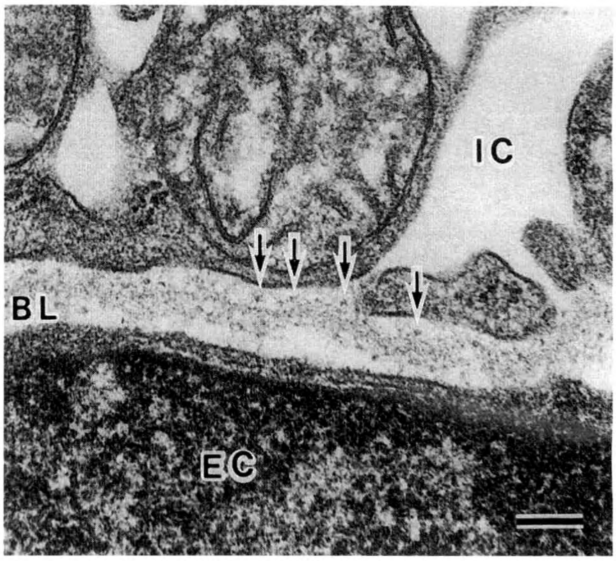


Fig 5. Reduced PEI distribution (arrows) on capillary BL in stria vascularis in treated animal. IC — intercellular space, EC — endothelial cell, bar — 0.2 µm.

and the endolymphatic sac after systemic administration of furosemide. Intravenous furosemide injection was used in order to ensure transport to the inner ear at as constant a rate as possible. Physiological saline solution adjusted to pH 7.4 in the same volume as the furosemide was administered to the control animals.

Schrott et al[15] demonstrated that guinea pigs displayed an advanced and severe edema in the stria vascularis at 10 to 30 minutes after 80 mg/kg furosemide injection. Mori et al[16] reported that furosemide (100 mg/kg) decreased the endocochlear potential to a negative level approximately 15 to 20 minutes after the start of the injection. We observed PEI distribution on the BL anionic sites in the labyrinth 20 minutes after the 80-mg/kg furosemide injection. We also noted severe enlargement of intercellular spaces and shrinkage of the intermediate cells in the stria vascularis, suggesting that systemically administered furosemide acts on the inner ear.

A change in PEI distribution is thought to reflect a change in the BL anionic sites of the labyrinth.[3,4,6] The PEI area in the strial vessels of the animals treated with furosemide was significantly decreased compared with that of the control animals treated with physiological saline solution. This difference suggests that the decrease in BL anionic sites in the strial vessels of the guinea pig is due to the furosemide injection. It has been reported that a change in blood pH or the administration of such cationic drugs as

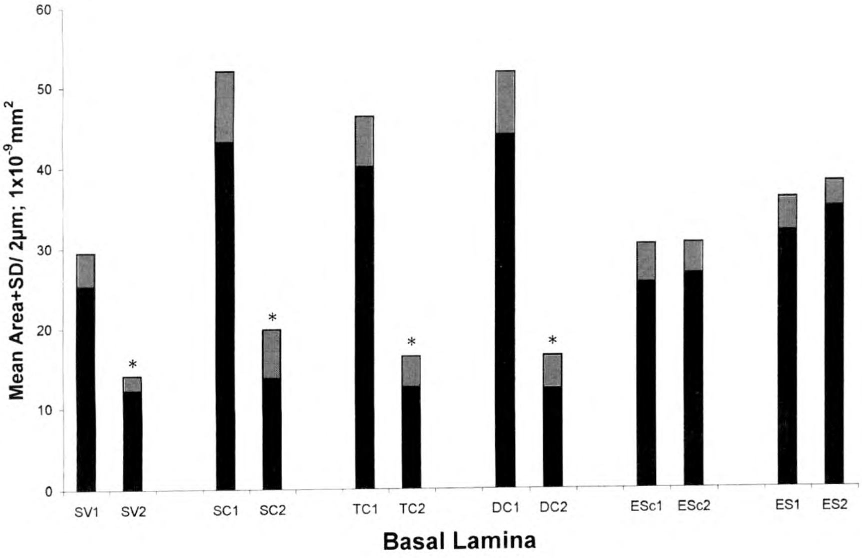


Fig 6. PEI area on basal lamina in control (1) and treated (2) groups. SV — stria vascularis; SC — sensory cell; TC — transitional cell; DC — dark cell area; ESc — capillary of endolymphatic sac; ES — endolymphatic sac; black bar — mean; gray bar — standard deviation; asterisk — $p < .05$, significantly different from control group.

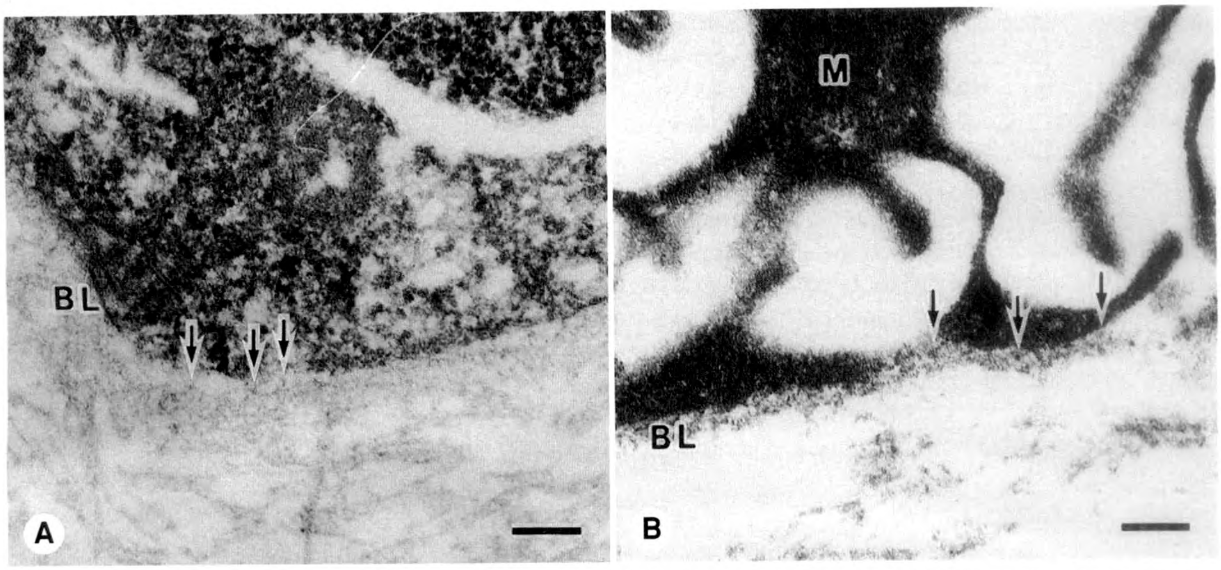


Fig 7. Decreased PEI distribution (arrows) on subepithelial BL in A) sensory cell area and B) dark cell area in treated animal. M — mitochondria, bars — 0.2 μm.

cisplatin or kanamycin[3,4,6] alters the BL anionic sites in the strial vessels of guinea pigs. Because furosemide is a negatively charged agent (pH 8.6 to 9.6), its charge cannot decrease the electrification on the capillary BL. In addition, the osmotic pressure of the furosemide solution is similar to that of physiological saline solution. A possible explanation for the decrease in BL anionic sites in the present study would be the pharmacological effects of furosemide. Marginal cells are thought to have a potassium (K^+) ion concentration as high as that in endolymph.[17] The K^+ transport in the marginal cells is mediated by the basolateral localization of Na^+, K^+-ATPase and the electrodiffusion across the luminal membrane.[17] Wangemann et al[18] reported that Na^+, K^+-ATPase and the Na-K-Cl co-transporter exist in the basolateral membrane, which is involved in the uptake of K^+ in marginal cells. It has also been demonstrated that the Na-K-Cl co-transporter localizes on the basolateral membrane infolding of the marginal cells and the membrane of the vascular endothelial cells in the stria vascularis.[19] Furosemide is thought to inhibit the function of the Na-K-Cl co-transporter and Na^+, K^+-ATPase.[20] Hence, the electrical neutralization on the capillary BL in the stria vascularis that occurred in the present study may be associated with a disturbance of the K^+ or sodium (Na^+) ion transport system in the basolateral membrane infolding of the marginal cells or in the membrane of the vascular endothelial cells in the stria vascularis.

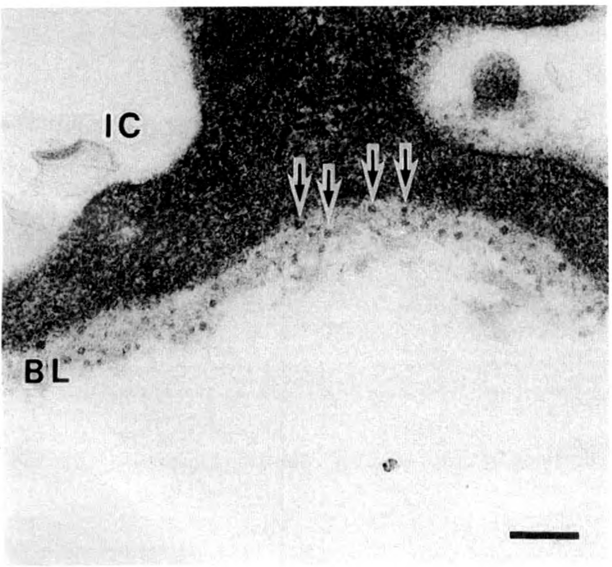


Fig 8. Endolymphatic sac with enlarged intercellular space (IC) in treated animal. PEI particles (arrows) are regularly distributed on subepithelial BL in endolymphatic sac. Bar — 0.2 μm.

In the dark cell area, the PEI distribution in the subepithelial BL in the treated animals was significantly decreased in comparison to that in the control animals. This finding suggests that the reduction of the BL anionic sites in the dark cell area was due to furosemide. It has been demonstrated that immunohistochemical staining for Na^+, K^+-ATPase is positive in the vestibular dark cell area.[21] With a monoclonal antibody, heavy immunolabeling of the Na-K-Cl co-transporter was demonstrated in the basolateral plasma membrane in the vestibular dark cell area.[22] The reduction of the BL anionic sites may be associated with the presence of Na^+, K^+-ATPase or the Na-K-Cl co-transporter in the dark cell area similar to that in the stria vascularis.

In transitional and sensory cells, the anionic sites in the subepithelial BL were significantly reduced in the furosemide-treated animals. The presence of the Na-K-Cl co-transporter was also detected in the subpopulation of fibrocytes underlying the vestibular neurosensory epithelium, apparently along their entire plasmalemma.[22] Reduction of BL anionic sites may be attributed to a defect of the Na-K-Cl co-transporter, which may have been caused by the furosemide administration. Ono and Tachibana[23] concluded that the sensory epithelium in the ampulla plays an important role in the maintenance of the ampullar endolymphatic potential. It has been reported that the ampullar endolymphatic potential does not change after furosemide administration.[23,24] Hence, we suggest that the change of the subepithelial BL anionic sites in the sensory cell does not affect the maintenance of the ampullar endolymphatic potential, at least in the early stage.

Morgenstern et al[25] found that intercellular edema occurred in the rugose portion of the endolymphatic sac after ethacrynic acid administration. A similar change was observed in the intercellular spaces of the endolymphatic sac in the present study. In the capillary BL and subepithelial BL in the endolymphatic sac, the PEI density and area in the treated animals were not significantly different from those in the control animals. This finding suggests that systemically administered furosemide does not affect the capillary and subepithelial BL anionic sites of the endolymphatic sac. Ichimiya et al[21] reported that immunostaining of Na^+, K^+-ATPase was mildly positive in the epithelial cells in the rugose portion of the endolymphatic sac and weakly positive in the subepithelial tissues of that portion. These anionic sites may not be too much influenced by the inhibition of Na^+, K^+-ATPase, which may be caused by the administration of furosemide.

In the present study, the enlargement of the intercellular spaces was observed in the intermediate cells of the stria vascularis. Crouch et al[22] found the presence of abundant Na-K-Cl co-transporter, which is a basolateral secretory isoform of the co-transporter, in the basolateral membrane of the strial marginal cells. The defect of the Na-K-Cl co-transporter due to furosemide administration may cause the inhibition of water transport into the scala media, inducing edematous changes in the stria vascularis. It has been demonstrated that reduction of the BL anionic sites may occur with an increase in capillary permeability in the labyrinth.[2] The reduction of capillary BL anionic sites that is induced by furosemide administration may facilitate the edematous changes in the stria vascularis.

In the present study, anionic sites were reduced in the capillary BL of the stria vascularis and the subepithelial BL in the sensory, transitional, and dark cell areas in the ampullar crista after the injection of furosemide. These findings suggest that the negative charge barrier in these areas may be disrupted by acute furosemide administration. Rybak[26] suggested that the combination of kanamycin and furosemide results in increased ototoxicity. He reported that the kanamycin concentration in serum and perilymph was significantly elevated in animals treated with kanamycin and furosemide as compared to animals treated with kanamycin alone. When a combination of kanamicin and furosemide is administered, the injured negative charge barrier that may be induced by furosemide may alter the macromolecular transport in the labyrinth, allowing kanamycin to leave the blood and pass into perilymph.

ACKNOWLEDGMENT — The authors thank Yoshiro Mori for his technical assistance in electron microscopy.

REFERENCES

1. Juhn SK, Rybak LP. Labyrinthine barriers and cochlear homeostasis. Acta Otolaryngol (Stockh) 1981;91:529-34.

2. Suzuki M, Kitamura K, Nomura Y. Influence of changed blood pH on anionic sites in the labyrinth. Acta Otolaryngol (Stockh) 1995;115:747-53.

3. Suzuki M, Kaga K. Effect of cisplatin on the basement membrane anionic sites in the ampulla, macula, and stria vascularis of guinea pigs. Ann Otol Rhinol Laryngol 1997;106:971-5.

4. Yamasoba T, Suzuki M, Kaga K. Influence of chronic kanamycin administration on basement membrane anionic sites in the labyrinth. Hear Res 1996;102:116-24.

5. Tomoda K, Maeda N, Kumazawa T. An anionic charge barrier in the guinea pig cochlea. Eur Arch Otorhinolaryngol 1988;245:307-10.

6. Suzuki M, Yamasoba T, Kaga K. Effects of chronic administration of kanamycin on the basement membrane anionic sites in the crista ampullaris of guinea pigs. Laryngoscope 1998; 108:81-6.

7. Takumida M, Bagger-Sjöbäck D, Rask-Andersen H. The basement membrane and associated structures in the murine endolymphatic sac. Eur Arch Otorhinolaryngol 1988;245:266-72.

8. Iurato S. Planum semilunatum and dark cells. In: Iurato S, ed. Submicroscopic structure of the inner ear. Oxford, England: Pergamon Press, 1967:219-25.

9. Kimura RS. Animal models of endolymphatic hydrops. Am J Otolaryngol 1982;3:447-51.

10. Pike DA, Bosher SK. The time course of the strial changes produced by intravenous furosemide. Hear Res 1980;3:379-89.

11. Arnold W, Nadol JB, Weidauer H. Ultrastructural histopathology in a case of human ototoxicity due to loop diuretics. Acta Otolaryngol (Stockh) 1981;91:399-414.

12. Rybak LP. Ototoxicity of loop diuretics. In: Rybak LP, guest ed. Ototoxicity. Otolaryngol Clin North Am 1993;26:829-44.

13. Futaki T, Kitahara M, Morimoto M. A comparison of the furosemide and glycerol tests for Meniere's disease. With special reference to the bilateral lesion. Acta Otolaryngol (Stockh)

1977;83:272-8.

14. Schurer JW, Kalicharan D, Hoedemaeker PHJ. The use of polyethyleneimine for demonstration of anionic sites in basement membranes and collagen fibrils. J Histochem Cytochem 1978;26:688-9.

15. Schrott A, Egg G, Lichtenberger O, Ernst A, Mest HJ. The time-course of furosemide-induced strial changes in guinea pigs after pretreatment with daltroban. Hear Res 1989;42:17-22.

16. Mori N, Uomizu K, Yura K, Sakai S. The difference in endocochlear and endolymphatic sac d.c. potentials in response to furosemide and canrenoate diuretics. Eur Arch Otorhinolaryngol 1990;247:371-3.

17. Melichar I, Syka J. Electrophysiological measurements of the stria vascularis potentials in vivo. Hear Res 1987;25:35-43.

18. Wangemann P, Liu J, Marcus DC. Ion transport mechanisms responsible for K^+ secretion and the transepithelial voltage across marginal cells of stria vascularis in vitro. Hear Res 1995;84:19-29.

19. Mizuta K, Adachi M, Iwasa KH. Ultrastructural localization of the Na-K-Cl cotransporter in the lateral wall of the rabbit cochlear duct. Hear Res 1997;106:154-62.

20. Ikeda K, Oshima T, Hidaka H, Takasaka T. Molecular and clinical implications of loop diuretic ototoxicity. Hear Res 1997;107:1-8.

21. Ichimiya I, Adams JC, Kimura RS. Immunolocalization of Na^+, K^+ ATPase, Ca^{++}-ATPase, calcium-binding proteins, and carbonic anhydrase in the guinea pig inner ear. Acta Otolaryngol (Stockh) 1994;114:167-76.

22. Crouch JJ, Sakaguchi N, Lytle C, Schulte BA. Immunohistochemical localization of the Na-K-Cl co-transport (NKCC1) in the gerbil inner ear. J Histochem Cytochem 1997;45:773-8.

23. Ono T, Tachibana M. Origin of the endolymphatic DC potential in the cochlea and ampulla of the guinea pig. Eur Arch Otorhinolaryngol 1990;248:99-101.

24. Kusakari J, Thalmann R. Effects of anoxia and ethacrynic acid upon ampullar endolymphatic potential and upon high energy phosphates in ampullar wall. Laryngoscope 1974;84:132-47.

25. Morgenstern C, Miyamoto H, Arnold W, Vosteen KH. Functional and morphological findings of endolymphatic sac. Acta Otolaryngol (Stockh) 1982;93:187-94.

26. Rybak LP. Furosemide ototoxicity: clinical and experimental aspects. Laryngoscope 1985;95(suppl 38).

Ann Otol Rhinol Laryngol 110:2001

IMAGING CASE STUDY OF THE MONTH

RHINOSCLEROMA MIMICKING NASAL POLYPOSIS

MAHMOUD EL-SHERIF M. AMMAR, MD
ARIE ROSEN, MD
NEWARK, NEW JERSEY

INTRODUCTION

Rhinoscleroma (RS) is seen with increasing incidence in the United States in a sporadic distribution. Most affected individuals are immigrants from endemic regions. It is a rare, slowly progressing granulomatous disease caused by *Klebsiella rhinoscleromatis*. The nose is invariably involved. Other areas of the upper airway, such as the larynx, pharynx, and trachea, are involved in 15% to 80% of cases. In the early stage, patients may experience catarrhal symptoms resembling nonspecific rhinitis. In the advanced stage, the appearance of a nasal mass or extensive crusting with epistaxis may be mistaken for other conditions, such as a nasal tumor or atrophic rhinitis. Because of the rarity of the disease in the United States, RS is often overlooked in the differential diagnosis of patients with these symptoms. Early recognition is important because of the potential for extensive destruction and spread to other individuals. Information on the distinctive radiologic appearance of RS is scarce, so that preoperative diagnosis of nasal RS is difficult. The computed tomography (CT) findings are not specific, but can be suggestive of the diagnosis in some cases. In the case presented here, RS was confused clinically with nasal polyps. Specific distinctive differential radiologic and clinical characteristics of RS are emphasized. Surgical debridement and prolonged administration of antibiotics constitute the mainstay of therapy.

CASE REPORT

A 26-year-old Central American male immigrant presented with a 1-year history of progressive bilateral nasal obstruction and fetid rhinorrhea. He tested negative for human immunodeficiency virus (HIV) and was otherwise healthy. On physical examination, bilateral fleshy polypoid nasal masses obstructing his nasal passages were noted. He had no external nasal deformity. A CT scan (Fig 1A) revealed extensive soft tissue densities involving both nasal cavities and the right maxillary sinus. These findings corroborated our clinical impression of nasal polyposis. On endo-

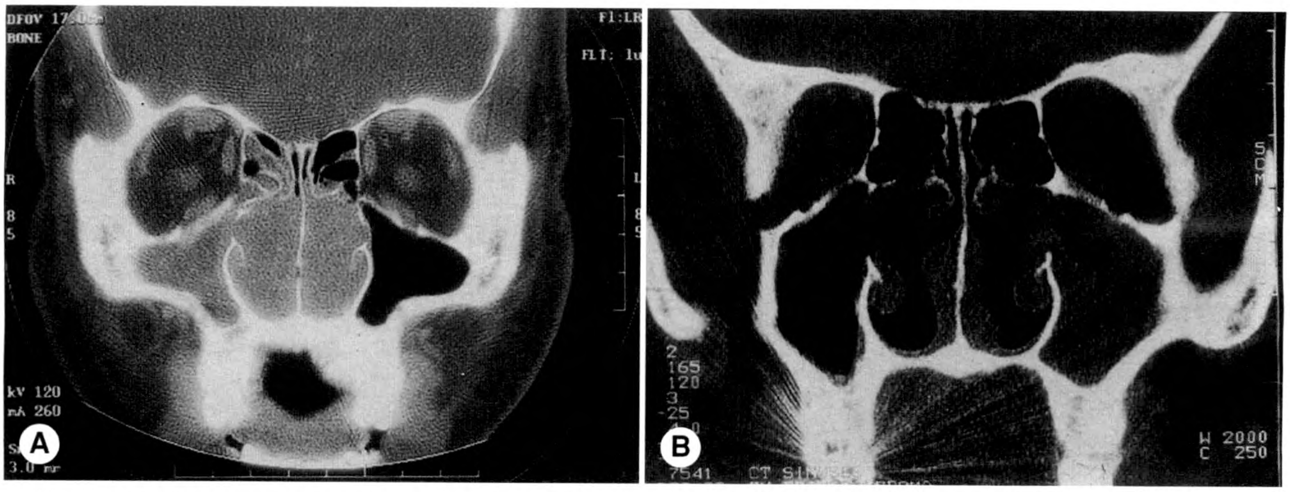


Fig 1. Computed tomographic scans of patient with rhinoscleroma. A) Preoperative. B) Postoperative, after 6 months of ciprofloxacin hydrochloride therapy.

From the Division of Otolaryngology, Department of Surgery, University of Medicine and Dentistry of New Jersey–New Jersey Medical School, Newark, New Jersey.

Presented as a poster at the meeting of the American Academy of Otolaryngology–Head and Neck Surgery, New Orleans, Louisiana, September 26-29, 1999.

CORRESPONDENCE — Arie Rosen, MD, UMDNJ–New Jersey Medical School, Division of Otolaryngology, Dept of Surgery, Medical Science Building, Room H592, 185 S Orange Ave, Newark, NJ 07103.

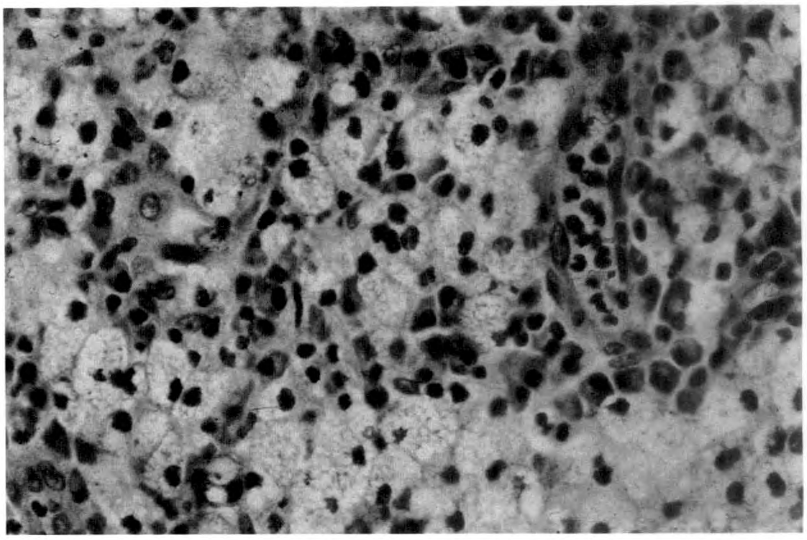


Fig 2. Histologic micrograph shows chronic inflammatory infiltration of monocytes, lymphocytes, and Mikulicz cells (vacuolated histiocytes with peripheral nuclei; original ×40).

scopic excision, the nasal masses were noted to be dense, arising from and uncharacteristically adherent to the nasal septum on both sides. The middle meatuses were free of disease. Only trapped secretions were found in the right maxillary sinus. An extensive histiocytic and lymphoplasmacytic infiltrate was noted on histologic examination; a culture of this infiltrate grew *K rhinoscleromatis*. Prolonged administration of ciprofloxacin hydrochloride, nasal irrigations, and frequent crust removal in the clinic were initiated after the operation. At 6 months, a CT scan showed only maxillary sinus stasis without disease progression (Fig 1B).

DISCUSSION

Epidemiology. The recent increase in the incidence of RS in the United States has been attributed to immigration from endemic areas. Endemic geographic locales include Eastern and Central Europe, Central and South America, East Africa, and the Indian subcontinent. Young adults in their second and third decades are most commonly affected, although the disease may be found in all age groups. Airborne disease transmission requires prolonged contact, in combination with predisposing factors such as poor hygiene, poor nutrition with a possible association of iron deficiency, and crowded living accommodations. These conditions characteristically exist in populations with low socioeconomic status.[1] There have also been a few reports of RS in HIV-infected individuals.[2]

Clinical Manifestations. Rhinoscleroma can affect the upper and lower airways and has a strong predilection for the nose. Most patients present with manifestations of advanced disease, including nasal obstruction (94%), nasal deformity (32%), and epistaxis (11%). The nose is involved in almost all cases, yet the reported rate of sinus involvement is low. The paucity of sinus involvement in the face of extensive nasal disease is striking in the case presented. In a study of 80 patients with RS, the nose was involved in all patients, the maxillary sinus in only 22%, the larynx in 26%, the trachea in 20%, and the eustachian tube in 27%.[3] The pharynx is involved in 18% to 43%.[3] Other structures of the face and respiratory system are rarely affected.

Rhinoscleroma has 3 clinical stages of disease progression. Nasal obstruction and purulent and fetid rhinorrhea lasting weeks to months characterize the initial, catarrhal-atrophic stage. Crusting and atrophy of the nasal mucosa can also be present. Laryngeal manifestations include hoarseness with interarytenoid hyperemia, exudates, and vocal fold edema. In the second, granulomatous stage, nonspecific symptoms such as epistaxis, nasal obstruction, and anosmia are common. Rubbery granulomas may present as a nasal mass. With progression, pressure atrophy from expanding granulomas may occur, leading to various degrees of destruction of the nasal skeleton. In the larynx, glottic and subglottic granulomas can cause airway narrowing with impaired vocal cord mobility. The final, sclerotic stage is characterized by extensive scarring causing nasal stenosis and external deformity. In the larynx, progressive scarring may lead to glottic and subglottic stenosis with subsequent stridor and airway obstruction.[4]

Pathophysiology. The disease process is believed to begin at the junction between two normal or metaplastic epithelia in the nose, larynx, or pharynx. The epithelial metaplasia seen with iron deficiency may be the basis for its predisposition to cause RS.

The chronic stage of RS is the more pathologically distinctive; histiocytic granulomas are evidence of ineffective phagocytosis of the encapsulated organism by macrophages (Fig 2). There is often pseudoepitheliomatous hyperplasia and an abundance of

Mikulicz cells (vacuolated large histiocytes with a peripheral nucleus) in the submucosal layer. The combination of Mikulicz cells and gram-negative intracellular organisms is suggestive of the diagnosis.

The humoral immunity in affected individuals is intact. An increased IgA:IgG ratio has been reported with normalization after therapy and can potentially provide an indicator of treatment response and disease reactivation. Acquired deficiencies in cellular immunity have been documented in individuals with RS. These include a decreased CD4 lymphocyte count, a decreased response of CD4 lymphocytes to interleukin-2 stimulation, and a decreased CD4:CD8 ratio. Reports of RS in HIV-infected individuals have led to the suggestion that this disease be considered an opportunistic infection in this subgroup of patients.[5]

Diagnosis. The clinical presentation of RS is often nonspecific, mimicking that of other, more common nasal disorders. It should be suspected in patients with extensive nasal polyps adherent to the nasal septum who exhibit a disproportionate lack of sinus involvement. Operative endoscopy is both diagnostic and therapeutic. Nasal biopsies should be directed to areas of active disease: the septum and the inferior turbinates. Cultures are positive 50% to 60% of the time and are diagnostic, since *K rhinoscleromatis* is not present in the normal nasal flora.

On CT, the nose is universally affected. Lesions vary from localized mucosal thickening to obliterative soft tissue densities extending from the septum to the lateral nasal wall with bony nasal expansion. Lesions characteristically are homogeneous, do not enhance, and have well-defined borders. Sinus opacification may represent retained secretions or mucosal disease ranging from mucosal thickening to complete sinus obliteration. Bone and cartilage involvement is present in extreme cases. There may be complete erosion of the turbinates and medial maxillary wall and resorption of the septal cartilage due to pressure necrosis. Laryngeal RS has a predilection for the subglottis. On CT, it may manifest as an irregular circumferential narrowing of the subglottic lumen with extension down to the trachea. In the trachea, cryptlike irregularities may be seen. Other, uncommon radiologic manifestations of RS have also been described. These include laryngeal posterior web formation and orbital, intracranial, parapharyngeal, and infratemporal extension.[6]

Treatment. Rhinoscleroma is difficult to eradicate. Despite aggressive therapy, the relapse rate is high, since the organism can remain dormant in its spore form and be reactivated months to years later. Treatment should include a combination of surgical debridement and long-term antibiotic therapy. Surgical debridement is used to remove necrotic tissue and restore airway patency. For laryngeal involvement, the use of the CO_2 laser is beneficial because of its enhanced hemostasis and accuracy.[7] Nasal deformity may necessitate reconstruction once the disease is eradicated.

Tetracycline has traditionally been used to treat this disease. It is relatively inexpensive and is also effective against spore formation. However, it is contraindicated in children and pregnant women, and its dosing schedule (4 times daily) presents a compliance issue. Antibiotic therapy has evolved with the recent introduction of fluoroquinolones. Fluoroquinolones have excellent activity against gram-negative organisms, convenient oral dosing, and low toxicity. Of note, their efficacy against RS has been demonstrated only anecdotally at the present time. Months of antibiotic therapy are often needed until biopsy and culture results are negative.

SUMMARY

Rhinoscleroma is increasing in incidence in the United States. It should be considered in patients who are immigrants from endemic countries and present with nasal polyposis that exhibits significant adherence to the nasal septum and relative sparing of the sinuses. Fluoroquinolones are emerging as the adjunctive antibiotic treatment of choice to complement surgical extirpation of the disease.

REFERENCES

1. Adraca R, Edson RS, Kern EB. Rhinoscleroma: a growing concern in the United States? Mayo Clinic experience. Mayo Clin Proc 1993;68:1151-7.
2. Shum TK, Whitaker CW, Meyer RR. Clinical update on rhinoscleroma. Laryngoscope 1982;92:1149-55.
3. Gamea AM. Role of endoscopy in diagnosing scleroma in its uncommon sites. J Laryngol Otol 1990;104:619-21.
4. Stiernberg CM, Clark WD. Rhinoscleroma: a diagnostic challenge. Laryngoscope 1983;93:866-70.
5. Amoils CP, Shindo ML. Laryngotracheal manifestations of rhinoscleroma. Ann Otol Rhinol Laryngol 1996;105:336-40.
6. Abou-Seif SG, Baky FA, El-Ebrashy F, Gaafar HA. Scleroma of the upper respiratory passages: a CT study. J Laryngol Otol 1991;105:198-202.
7. Maher AI, El-Kashlan HK, Soliman I. Rhinoscleroma: management by carbon dioxide surgical laser. Laryngoscope 1990;100:783-8.

ANNALS

OF OTOLOGY,
RHINOLOGY &
LARYNGOLOGY

CHANGE OF ADDRESS

All address changes must be received within 45 days prior to the effective date. This will assure that your subscription will be received without interruption.

Send old and new addresses as early as possible to:

ANNALS PUBLISHING COMPANY
4507 LACLEDE AVENUE
ST LOUIS, MO 63108
(314) 367-4987
Fax (314) 367-4988
e-mail: manager@Annals.com

A14

Courses on the Lateral Approaches to the Skull Base
(*In English*)

Course Director:
Mario Sanna, MD

This cadaver hands-on workshop provides the individual opportunity to participate in:

- Video presentation of techniques and clinical cases
- Didactic presentations
- Performing the surgical procedures on specially prepared bones and half-heads
- Discussions

For additional information, contact:

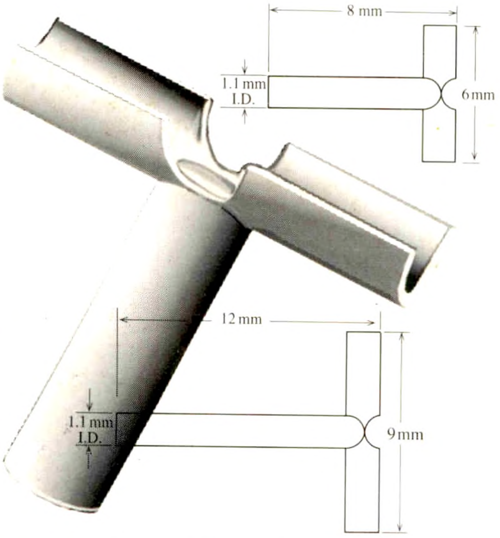
Secretary Gruppo Otologico
Via Emmanueli n.42
29100 Piacenza - Italy
telephone: 0523/754362
fax: 0523/453708
E-mail: Corsi.Sanna@otologic.it

A161

Surgeon Controlled™
SuperSoft Silicone™ T-Tubes

*The ideal situation where intubation time is determined by the Otologist, rather than the vagaries of spontaneous extrusion.**

Introduced by Santa Barbara Medco, Inc. The SuperSoft Silicone T-Tube has over 17 years of successful clinical experience as a surgeon controlled ventilation tube. Available in Pretrimmed and Standard sizes.



Advantages of the SuperSoft Silicone T-Tube (SSTT):*

- The surgeon controls the intubation period for the large majority of patients.
- Low spontaneous extrusion rate - less than 10% when flanges are trimmed to 3mm.
- Reported lower residual perforations rate (6.7%) than other long-term ventilation tubes.
- Tube removal can be simply and safely performed in the office because of the shortened SuperSoft Silicone flanges.

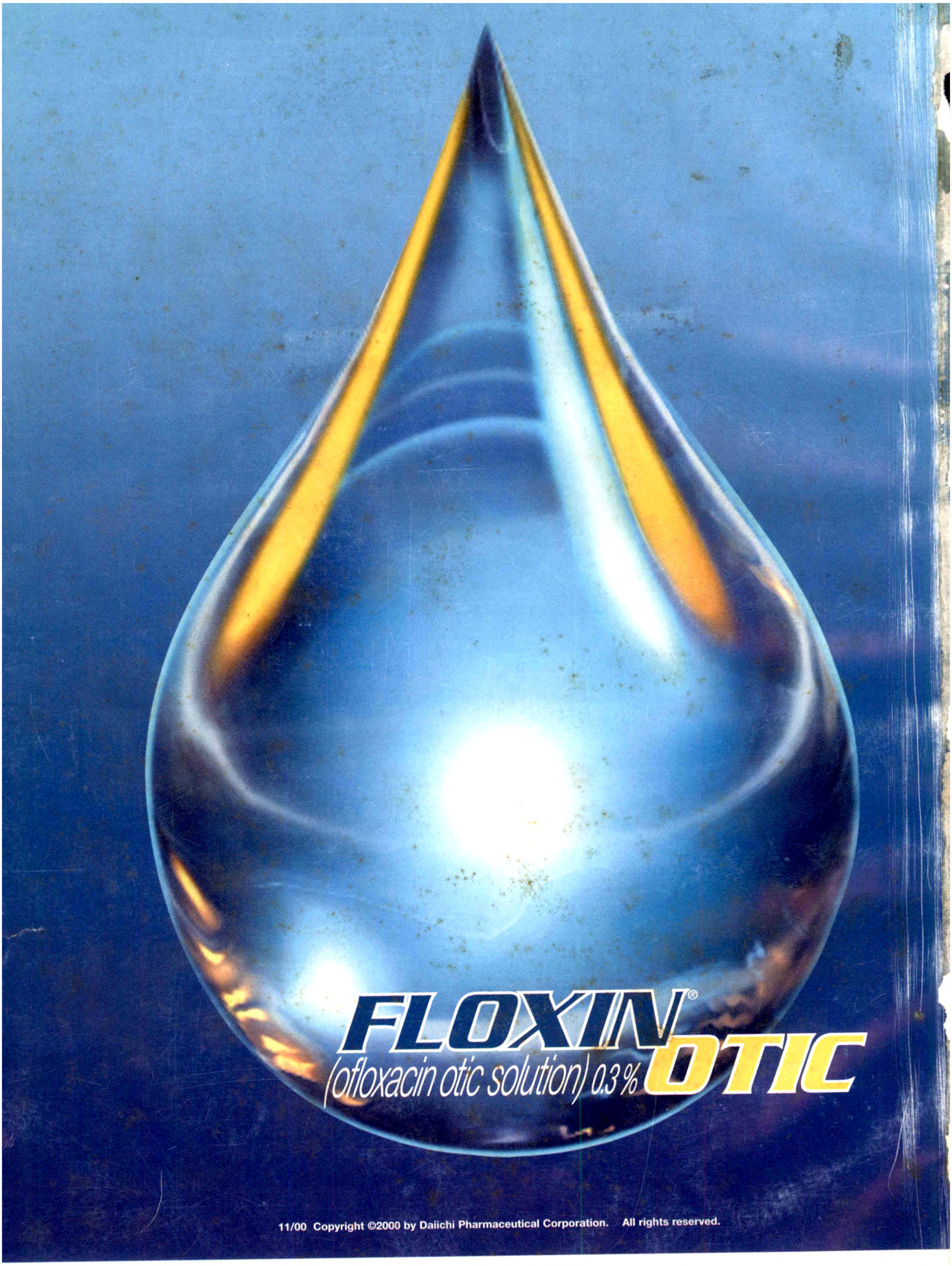
Please contact us for additional information and an evaluation sample.

Santa Barbara Medco, Inc.

P.O. Box 6843
Santa Barbara, CA 93160
805/683-1486
FAX 805/683-4864

e-mail: sbmedco@silcom.com or
visit us at our website www.sbmedco.com

*"Lowering the Incidence of Perforation following Goode T-Tube Removal", presented at the American Society of Pediatric Otolaryngology Annual Meeting, May 19, 1990, Toronto, Canada, A.F. Inglis, Jr., M.D., M.A. Richardson, M.D., Taya S. Higgins, R.N. - Children's Hospital & Medical Center, 4800 Sandpoint Way, N.E., P.O. Box C5371, Seattle, WA 98105



11/00 Copyright ©2000 by Daiichi Pharmaceutical Corporation. All rights reserved.

Please see our full ad on pages 214a-d.